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MATE PREFERENCE IN MALES OF FOUR SPECIES OF GAMBUSIINE FISHES[1]

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Fishes have evolved many methods of preventing hybridization. Ethologic factors are very important in reproductive isolation of most species (Hubbs, 1955; Gordon, 1957). The most obvious involve mate preference. Unfortunately too few projects have been designed with a specific goal of studying mate selection. Most of those are on the poeciliid fishes, which are specialized in that the eggs are fertilized within the female. Observations on egg-laying etheostomatine fishes also indicate the importance of mate selection among these fishes (Hubbs, in press).

Previous studies on poeciliid courtship (Haskins and Haskins, 1949; Clark, Aronson, and Gordon, 1954) show that females are more or less passive in courtship and that males play the major role in mate selection. Gambusiine females are also relatively passive in courtship (Warburton, Hubbs, and Hagen, 1957), but are more likely than most other poeciliid females to try to repulse over-active males. This study is based on the analysis of male courtship behavior in four species of *Gambusia* and a hybrid.

METHODS

Males were isolated in individual aquaria of approximately one-gallon capacity. The males were placed in the one to two-gallon observation tanks at least 24 hours before the females were introduced. The observations were carried out over the following 30 minutes after which the females were removed. The male was not presented with other females for at least 24 hours. The females were isolated in five-gallon aquaria between experiments. All fish used were reared in the laboratory. Except for the hybrid males, no fish had experience with members of other species prior to the experimental interval. No male was exposed twice to the same female.

Either a single female or two females of different species were placed with the male. The same patterns of behavior were noted but selection was most pronounced when two females were used. Thereby, detailed data on single female experiments are not reported. Similar to the results obtained by Clark, Aronson, and

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Gordon (1954), copulation frequency was reduced in the presence of an additional female. However, since isolated pairing is not a natural phenomenon, copulation in nature must occur in the presence of other individuals. In two female experiments, equal-sized females were used except in those contrasting *Gambusia geiseri* and *G. heterochir*. An indication of preference for the larger females was noted, especially when males of the other species were used, which may account for the apparent preference of swordtail females (larger) over platyfish females (smaller) by platyfish males reported by Clark, Aronson, and Gordon.

Both Haskins and Haskins (1949) and Clark, Aronson, and Gordon (1954) have noted changes in courtship activities that correlate with the experience of the fish. Haskins and Haskins showed that male courtship was more discriminating after exposure to the females for a short interval. Clark, Aronson, and Gordon showed that male courtship was directed more toward females of the species with which he had been held for a long interval than toward females he had not courted. Therefore, the courtship activity of poeciliine fishes clearly shows the effects of the past experience of the fishes. In the related gambusiine fishes studied here; however, experience seems to have less significance. No trend can be noted in the activity of males presented with females of the same species on subsequent days. Therefore, the behavior of a single male remains at the same level as he becomes more experienced with any kind of female both in single matings and in paired matings.

Of the behavioral activities listed by Clark, Aronson, and Gordon (1954), nibbling, thrusting, and swinging are reported in this paper. Thrusting is here restricted to those anteriorly directed movements of the gonopodium that are obviously directed at the genital aperture of the female. Swinging is here considered to be an anteriorly directed movement of the gonopodium not directed toward the genital area of the female; however, it is carried out in the near proximity of that female. Nondirected swinging is that activity carried out far from the females or at an intermediate distance between them so that it is not possible to ascertain toward which female the action is directed. This classification somewhat differs from that of previous authors. Other activities indicate mate preference. Some, such as copulation, are not reported in detail because they are relatively infrequent. Others, such as pecking, were not recorded because of the difficulty of determining toward which female the behavior was directed.

Fourteen copulations have been recorded during thirteen of the observations intervals. Eleven were homospecific and three were between species. In the pertinent intervals the courtship activity of the male was approximately that of the same male during other experiments, due to a high activity prior to copulation and a low activity afterwards. These data are not sufficient to show statistically reliable mating preference, but do indicate that in *Gambusia* the relative amounts of the courtship activities are directly correlated with copulation frequency. Clark, Aronson, and Gordon noted that courtship may be inversely correlated with copulation frequency in intraspecific matings; however, their tables 41 and 42 show a correlation between courtship activity and copulations when inter- and intraspecific matings are compared.

Species Relationships

Four species of *Gambusia* have been used in these experiments. Their morphologic relationships and ecologic preferences are listed below:

Gambusia affinis (Baird and Girard) is widely distributed in the southeastern United States and is of almost catholic ecology. It has been widely used for mosquito control and is now virtually cosmopolitan in tropical and temperate fresh-waters (Krumholtz, 1948). The

western limits of its natural range pass through Texas. Here it occupies a variety of habitats, except where other species of *Gambusia* abound.

Hubbs (1926) separated *G. affinis* (using the synonyms *G. holbrooki* and *G. patruelis*) subgenerically from other species of *Gambusia*. No other species of *Gambusia* has been placed in the same subdivision with *G. affinis*.

The five males and 30 females used were reared from stocks collected in Austin. No other gambusiine fishes are known to occur there.

Gambusia heterochir Hubbs is restricted to the headwaters of Clear Creek, Menard County, Texas, where it hybridizes extensively with *G. affinis* (Hubbs, 1957). Like many other gambusiine fishes of the Southwest and Mexico, it is restricted to clear limestone spring waters (Hubbs, 1959). This restriction may be due to preference or to an inability to compete with *G. affinis* elsewhere. The latter hypothesis is the more probable, as Warburton (1958) succeeded in maintaining pure stocks of *G. heterochir* elsewhere but could not maintain *G. heterochir* when mixed with *G. affinis*.

Hubbs (1957) considered *G. heterochir* to be related to *G. nicaraguensis* Günther and thus distantly related to *G. affinis*.

The nine males and 28 females used were obtained from stocks reared from fish collected at Clear Creek. *Gambusia affinis* is the only other gambusiine fish there.

Gambusia geiseri Hubbs and Hubbs is restricted to clear springs in Texas (Hubbs and Springer, 1957). Its natural range appears to be limited to San Marcos and Comal springs. This restriction is probably due to its inability to compete with *G. affinis* downstream.

Hubbs (1926) placed this species, which he then erroneously called *G. affinis*, in the *G. nobilis* group. The *G. nobilis* and *G. nicaraguensis* species groups are usually considered to be closely related.

The three males and 14 females used were reared from stocks collected in Toyahvale, Texas, from a stock probably introduced there from San Marcos. *Gambusia geiseri* is sympatric with *G. affinis* over most of its range, however, only *G. nobilis* occurs with *G. geiseri* at Toyahvale. *G. geiseri* is not known to hybridize with *G. affinis* or *G. nobilis*.

Gambusia hurtadoi Hubbs and Springer is restricted to a spring, Ojo de la Hacienda Dolores, in Chihuahua, Mexico. Its distribution is now limited by dry stream bed (Hubbs and Springer, 1957). Undoubtedly it once contacted *G. senilis* Girard. It is doubtful, however, that the species has ever been sympatric with *G. affinis*.

This species has been placed in the *G. nobilis* group with *G. geiseri*.

The five males and 16 females used were reared from stocks obtained from the Ojo and maintained in the laboratory for three or more generations. No other species of gambusine fishes now inhabits the collection locality. *G. hurtadoi* is not known to hybridize with other gambusiine fishes except under experimental conditions.

Mate Preferences

Males of *Gambusia affinis* exhibit a clear homospecific preference when *G. affinis* females are contrasted with either *G. hurtadoi* or *G. geiseri* (table 1). No such preference is noted, however, when *G. heterochir* and *G. affinis* females are contrasted. Likewise, if only one female is used, *G. affinis* males court *G. affinis* and *G. heterochir* females more avidly than they court *G. hurtadoi* or *G. geiseri* females. Copulation between a *G. affinis* male and *G. heterochir* female further indicates the lack of mate recognition between these species. These data indicate that (1) enough *G. affinis* genes have introgressed into the *G. heterochir* populations to obscure the recognition; (2) *G. affinis* and *G. heterochir* are much more closely related than is indicated by their gonopodial morphology. The first alter-

TABLE 1. *Mate preferences by* G. affinis *males exposed to paired females. The figures given are the average number of times that the stated activity was performed per 30 minute observation trial. The probabilities of significance of the differences (p) were derived using Snedecor's (1946) formulae for the comparison of two groups*

	Nibbles	p	Thrusts	p	Swings	p	Non-directed swings	Trials
G. heterochir ♀	0.65	0.2	3.47	0.8	5.52	0.4	5.70	17
G. affinis ♀	1.41		3.00		8.29			
G. geiseri ♀	0.00	0.009	0.00	0.002	0.43	0.001	1.50	6
G. affinis ♀	1.00		1.28		6.86			
G. hurtadoi ♀	0.90	0.3	6.50	0.07	13.20	0.04	4.80	10
G. affinis ♀	2.30		15.70		36.40			
G. geiseri ♀	2.00	0.6	7.00	0.3	22.67	0.4	8.13	6
G. heterochir ♀	2.83		18.67		37.50			
G. hurtadoi ♀	0.33	0.09	1.50	0.02	8.00	0.02	11.08	12
G. heterochir ♀	1.25		4.92		16.25			
G. hurtadoi ♀	0.00		0.12	0.3	2.50	0.2	7.75	8
G. geiseri ♀	0.00		1.00		5.00			

native has merit but is somewhat contradicted by the apparent scarcity of backcrosses with *G. heterochir* in the natural population studied by Hubbs (1957 and 1959). The abundance of apparent backcrosses with *G. affinis* is not germane as *G. affinis* stocks used here came elsewhere. The question of relationship of *G. affinis* to *G. heterochir* must remain unsettled, especially because correlation of mate preference with degree of phylogenetic relationship within genera has not yet been adequately documented. Although the individual tabula-

TABLE 2. *Mate preferences by* G. heterochir *males exposed to paired females. The figures given are the average number of times that the stated activity was performed per 30-minute observation trial. The probabilities of significance of the differences (p) were derived using Snedecor's (1946) formulae for the comparison of two groups*

	Nibbles	p	Thrusts	p	Swings	p	Non-directed swings	Trials
G. heterochir ♀	1.00	0.9	2.82	0.5	10.29	0.7	7.35	17
G. affinis ♀	1.11		2.06		12.71			
G. geiseri ♀	0.50	0.6	0.00	0.4	2.50	0.02	1.33	6
G. affinis ♀	0.33		0.50		19.83			
G. hurtadoi ♀	0.50	0.3	0.67	0.2	1.67	0.2	3.16	6
G. affinis ♀	1.17		1.50		10.83			
G. geiseri ♀	1.17	0.02	3.56	0.08	7.61	0.02	11.50	18
G. heterochir ♀	3.00		6.94		17.67			
G. hurtadoi ♀	0.25	0.2	1.83	0.2	9.50	0.007	12.58	12
G. heterochir ♀	0.75		3.50		21.17			
G. hurtadoi ♀	0.38		2.50	0.07	32.12	0.2	6.62	8
G. geiseri ♀	0.38		0.75		6.25			

TABLE 3. *Mate preference of a* G. heterochir ♀ × G. affinis ♂ *hybrid male exposed to paired females. The figures given are the average number of times that the stated activity was performed per 30-minute observation trial. The probabilities of significance of the differences (p) were derived using Snedecor's (1946) formulae for the comparison of two groups*

	Nibbles	p	Thrusts	p	Swings	p	Non-directed swings	Trials
G. heterochir ♀	0.75	0.4	0.00		0.75	0.2	4.37	8
G. affinis ♀	0.25		0.00		0.00			
G. geiseri ♀	0.00		0.00		0.00		0.00	3
G. affinis ♀	0.00		0.00		0.00			
G. hurtadoi ♀	0.00		0.00		0.33	0.4	1.33	3
G. affinis ♀	0.00		0.00		0.00			
G. hurtadoi ♀	0.00		0.00		0.00		1.17	6
G. heterochir ♀	0.00		0.00		0.00			
G. hurtadoi ♀	0.67	0.4	0.00		0.67	0.4	1.67	3
G. geiseri ♀	0.00		0.00		0.00			

tions are not statistically significant, the consistent preference of *G. affinis* males for *G. heterochir* females over females of either *G. hurtadoi* or *G. geiseri* is in accord with the indication that *G. affinis* males do not discriminate between females of *G. affinis* and *G. heterochir*.

G. affinis males may discriminate between *G. affinis* and *G. geiseri* females better than they do between *G. affinis* and *G. hurtadoi* females; however, courting activities are more numerous when *G. hurtadoi* females are used. As *G. affinis* is sympatric with *G. geiseri* and allopatric to *G. hurtadoi*, reinforcement of behavioral isolation is suggested. The apparent (but not statistically significant) perference for *G. geiseri* over *G. hurtadoi* by *G. affinis* is not in accord, however.

Males of *G. heterochir* prefer their own females over those of *G. hurtadoi* and *G. geiseri* (table 2). The individual tabulations are mostly of marginal significance but their consistancy increases the reliability of the indicated preference. Again, no preference is noted when reactions to *G. affinis* and *G. heterochir* females are contrasted. Furthermore, when males of *G. heterochir* are presented singly with single females, they court more avidly with those of *G. affinis* and *G. heterochir* than with those of the other species. Likewise, in mating with females of two species, *G. heterochir* males appear to prefer *G. affinis* females over females of *G. hurtadoi* and *G. geiseri*. The single copulation between a *G. heterochir* male and a *G. affinis* female indicates a lack of ability to distinguish between the species.

The experiments with *G. heterochir* males all have a relatively low probability of significance despite the large number of tests run. This may indicate a low ability to distinguish females. Such a phenomenon would be in accord with Hubbs' (1957) hypothesis that *G. heterochir* has long been geographically isolated from its near relatives. Similarly, this behavior may account for the original breakdown of isolating mechanisms to form the hybrid swarm. The single copulation between a *G. heterochir* male and a *G. hurtadoi* female further indicates a lack of ability of the males to distinguish between females.

The single hybrid male used in the experiments was essentially inactive sexually (table 3). About the only thing he did was to swing his gonopodium without directing the activity to either female. This individual was F_1 result-

TABLE 4. *Mate preferences by* G. geiseri *males exposed to paired females. The figures given are the average number of times that the stated activity was performed per 30-minute observation trial. The probabilities of significance of the differences (p) were derived using Snedecor's (1946) formulae for the comparison of two groups.*

	Nibbles	p	Thrusts	p	Swings	p	Non-directed swings	Trials
G. heterochir ♀	0.50	0.7	0.00		0.38	0.5	2.37	8
G. affinis ♀	0.75		0.00		0.12			
G. geiseri ♀	0.33	0.4	2.67	0.002	15.00	0.001*	1.33	3
G. affinis ♀	0.00		0.00		0.00			
G. hurtadoi ♀	0.00		0.00		2.33	0.5	6.67	3
G. affinis ♀	0.00		0.00		1.33			
G. geiseri ♀	7.17	0.7	0.67	0.3	15.33	0.2	10.33	6
G. heterochir ♀	4.83		0.00		1.17			
G. hurtadoi ♀	0.00		0.00		0.67	0.2	3.17	6
G. heterochir ♀	0.00		0.00		0.00			
G. hurtadoi ♀	0.25	0.2	0.00	0.4	0.25	0.001	5.25	4
G. geiseri ♀	3.50		0.25		16.25			

* Probability determined by chi-square as t test gave probability of $1/\infty$.

ing from a cross between a virgin female *G. heterochir* and a *G. affinis* male. He was of approximate equal age to the *G. heterochir* and *G. affinis* males used in the experiments. His gonopodium was clear at the start of the experiments; he did not grow during the tests; and his activity remained low throughout the time. Furthermore, like the controls, he had opportunity to observe the courtship of his father. Thereby, he had attained his full degree of sexual maturity. He

TABLE 5. *Mate preferences by* G. hurtadoi *males exposed to paired females. The figures given are the average number of times that the stated activity was performed per 30-minute observation trial. The probabilities of significance of the difference (p) were derived using Snedecor's (1946) formulae for the comparison of two groups*

	Nibbles	p	Thrusts	p	Swings	p	Non-directed swings	Trials
G. heterochir ♀	2.24	0.4	2.11	0.003	7.76	0.7	3.82	17
G. affinis ♀	1.53		0.53		9.53			
G. geiseri ♀	0.67	0.5	1.83	0.02	6.00	0.7	1.50	6
G. affinis ♀	0.33		0.00		7.83			
G. hurtadoi ♀	4.89	0.4	4.78	0.007	30.00	0.0002	6.22	9
G. affinis ♀	0.56		0.00		4.33			
G. geiseri ♀	3.00	0.9	8.72	0.6	1.31	0.4	6.21	13
G. heterochir ♀	3.15		10.54		2.00			
G. hurtadoi ♀	1.25	0.009	6.50	0.003	21.17	0.0001	6.16	12
G. heterochir ♀	0.17		0.42		3.92			
G. hurtadoi ♀	0.75	0.3	6.50	0.01	30.75	0.0001	3.62	8
G. geiseri ♀	0.25		0.12		3.25			

is the laboratory hybrid male portrayed in figure 4 of Hubbs (1959). Single female-preference experiments using a male of the reciprocal cross also showed low courtship activity.

Males of *G. geiseri* consistently prefer *G. geiseri* females over those of the other three species, although the preference over *G. heterochir* is not of reliable statistical significance (table 4). The lack of clear-cut significance may be due to the circumstance that females of different size were used. This hypothesis is supported by the single-female experiments, in which the *G. geiseri* females were courted most avidly and the *G. heterochir* females least avidly. The reactions toward *G. affinis* and *G. hurtadoi* females were intermediate.

G. geiseri males exhibit a very low courtship activity for females of other species, perhaps as a result of its extensive sympatry with *G. affinis* in its natural range.

If a *G. hurtadoi* female and one of either *G. affinis* or *G. heterochir* are presented together to a male of *G. geiseri,* most of his courting is directed toward the more closely related *G. hurtadoi.* The limited comparisons, however, are not of clear statistical significance.

Males of *G. hurtadoi* show a strong preference for their own females over those of the three other species (table 5). These preferences appear to be the strongest displayed by any of the males tested. Hubbs and Springer (1957) suggested that *G. hurtadoi* and *G. senilis* were in contact and did not hybridize before the intervening fields were irrigated. If so, the ability to discriminate must have been well developed to keep such closely related species apart. On this line of thought, it is not surprising that *G. hurtadoi* readily recognizes more distantly related species as foreign.

Males of *G. hurtadoi* also appear to discriminate among two foreign species. This is best developed in thrusts, in which activity *G. heterochir* and *G. geiseri* are preferred over *G. affinis.* Both have been suggested as being more closely related to *G. hurtadoi* than is *G. affinis.* A correlation of mate selection and phylogeny is thus indicated.

Conclusions

1. Males of most species of *Gambusia* court females of their own species far more than they court females of other species.

2. The only exceptions to the above are the males of *Gambusia affinis* and *G. heterochir,* which have not been shown to be able to distinguish between females of those two species. These two species exhibit the highest known incidence of natural interspecific hybridization of any gambusiine fishes.

3. In addition to the lack of pronounced mate recognition between *G. affinis* and *G. heterochir,* males appear to prefer females of closely related species over more distantly related females.

4. Two hybrid males between *G. affinis* and *G. heterochir* have shown a very low sexual activity.

5. Males of species thought to have been sympatric with closely related species are more discriminating than males thought to have been allopatric to their close relatives.

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THE PATTERN OF INTERSPECIFIC VARIATION IN THE GENUS *ORYZA:* ITS QUANTITATIVE REPRESENTATION BY STATISTICAL METHODS[1]

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Two cultivated and more than twenty wild species occur in the genus *Oryza*. As is well known, *O. sativa* L., which comprises many varieties with different characteristics, is the most important food crop in Asian countries. *O. glaberrima* Steud., another cultivated species, is grown within a rather limited area in Tropical West Africa. Among wild species, *O. perennis* Moench (found in almost all tropical countries of the world), *O. sativa* f. *spontanea* Roschevicz (of tropical Asian countries) and *O. breviligulata* A. Cheval et Roehr. (of tropical African countries) appear to be relatively closely related to those cultivated species, in the sense that they can be easily crossed with one another and with the cultivated forms, and their chromosomes pair in the F_1 almost normally. Other wild species may show a spectrum of genetic distances from the cultivated rice. The phylogenetic hierarchy of the genus, which is not yet established, attracts interest of rice students, and as a basis for approaching it, a re-evaluation of interspecific relationships is desired.

The first basic taxonomic study of the genus *Oryza* was reported by Roschevicz (1931). He identified 20 species, and after surveying the characters of herbarium specimens and the geographical distributions, divided them into four sections, *Sativae, Granulatae, Coarctatae* and *Rhynchoryza*. Recently, Chatterjee (1948) added several new species to the genus and wrote a revised key to the classification of the species, which was first written by Roschevicz. Besides these, there are contributions to this problem, which are, however, of relatively fragmentary or local nature (Roy 1921, Sasaki 1930, Backer 1946, etc.).

Most taxonomists expect that their systematic classification of organisms represents the phylogenetic hierarchy of the group. This may be true insofar as his conclusion is based on a comprehensive study of the pattern of existing variations. In practice, however, among many characters differentiating species or races, a few will be empirically chosen as representatives of the whole variation, often leading to conclusions based on subjective opinions of the investigators.

With the view to eliminating subjectivity from systematic classification of insect species, Michener and Sokal (1957) have devised a method to group species based on quantified degree of resemblance among them. Their computation starts from a matrix of correlations between species. The nuclei of assembly of species are then formed and the results are shown in a tree-like diagram suggesting the phylogenetic hierarchy of the group. In addition to this, Sokal (1958) has tried to use techniques of factor analysis for the same purpose, also starting from a matrix of interspecific correlations.

Following the lines drawn by these biometrical entomologists, the present authors have attempted to establish a quantified systematic classification of the genus *Oryza*. Sixteen species were investigated and records were taken for

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42 characters. The methods adopted were, in the first place, factor analysis, and secondly, the procedure devised by Sokal and Michener (1958). The results obtained were largely comparable with those empirically reached by former workers, but displayed a more detailed structure of interspecific relationships, suggesting the usefulness of these methods for looking into the phylogenetic make-up of a group.

Materials and Characters Investigated

At present, numerous strains of *Oryza* belonging to 18 different species, collected from their natural habitats or sent by various institutions to whom we are greatly obliged, are being preserved in the National Institute of Genetics, Misima, Japan. They are grown in a greenhouse or in short-day fields. Their Latin names were determined by Dr. T. Tateoka of the Institute with the original descriptions. Sixteen of those species, listed in table 1, were used for this study. One of them, *O. malabarensis*, is a wild species resembling *O. officinalis* Wall., found in the mountain regions of South India. Whether it is a good species or not is however undecided.

As also shown in table 1, nine of these species were each represented by two or more strains, while only one strain was available for each of the other seven. Thus, the total number of strains investigated was 31. *O. sativa* L. is known to be sub-divided into two (*Indica* and *Japonica*) or three (*Continental, Tropical-Insular* and *Temperate-Insular*) types (Oka 1953). Of the five strains of this species used, two were of the *Indica* or *Continental*, one was of the *Tropical-*

Table 1. *List of* Oryza *species studied*

No.	Name identified	Section by Roschevicz	2n	Distribution	Strains used
1	*O. sativa* L.	Sativae	24	Cultivated, worldwide	108, 414, 521, 563, 647
2	— *f. spontanea* Roschev.	Sativae	24	Tropical Asia	W0106, W0122
3	*O. perennis* Moench*	Sativae	24	Tropical countries, worldwide	W0032, W0149
4	*O. glaberrima* Steud.	Sativae	24	West Africa, cultivated	W0040, W0492
5	*O. breviligulata* A. Cheval et Roehr.	Sativae	24	West Africa to Sudan	W0009, W0042, W0049
6	*O. australiensis* Domin	Sativae	24	Northern Australia	W0008
7	*O. latifolia* Desv.	Sativae	48	Central and South America	W0019, W0020, W0047
8	*O. alta* Swallen	Sativae	48	Central and South America	W0017
9	*O. minuta* Presl.	Sativae	48	Malaya, Java & Philippines	W0016, W0045
10	*O. officinalis* Wall.	Sativae	24	Tropical Asia	W0002, W0006, W0012
11	*O. eichingeri* Peter.	Sativae	48	East Africa	W0015, W0043
12	*O. malabarensis*			South India (Malabar Distr.)	W0021
13	*O. granulata* Nees	Granulatae	24	Tropical Asia	W0003
14	*O. ridleyi* Hook.	Coarctatae	48	Malaya & Thailand	W0001
15	*O. brachyantha* A. Cheval et Roehr.	Coarctatae	24	West and Central Africa	W0023
16	*O. subulata* Nees	Rhyncoryza	24	South America	W0510

* *O. barthii* A. Cheval, *O. longistaminata* A. Cheval and *O. cubensis* Ekman are included in *O. perennis*.

Remarks: Important species absent in the materials studied are *O. grandiglumis* Prod. (South America), *O. punctata* Kotschy (East Africa), *O. meyeriana* Bail. (Java and Philippines), and *O. coarctata* Roxb. (India).

TABLE 2. *List of characters investigated*

No.	Character	State code or unit	Remarks
1	Ligule shape	3–2–1	Roundish—Acute
2	Ligule, hairiness	3–2–1	Haired—Hairless
3	Ligule, stiffness	3–2–1	Stiff—Membraneous
4	Auricle size	3–2–1	Large—Small
5	Leaf blade width	3–2–1	Broad—Narrow
6	Spreading of leaves	3–2–1	Spread—Erect
7	Spreading of tillers	3–2–1	Spread—Erect
8	Plant height	3–2–1	Tall—Dwarf
9	Internode septum	3–1	Present—Absent
10	Panicle number per stem	3–1	Two or more—One
11	Thickness of rachis	3–2–1	Thick—Thin
12	Bristles on rachis	3–1	Present—Absent
13	Panicle shape	3–2–1	Spread—Closed
14	Articulation of pedicel	3–2–1	Rectangular—Oblique
15	Empty lemma shape	3–1	Setaceous—Linear
16	Empty lemma, ciliatedness	3–1	Ciliated—Non-ciliated
17	Apical elongation of inner glume	3–2–1	Elongated—Non-elongated
18	Bristles distribution on glumes	3–1	Along ribs—On whole surface
19	Thickness of awn	3–2–1	Thick—Thin
20	Rhyzome development	3–2–1	Well developed—Absent
21	Duration of plant	3–1	Perennial—Annual
22	Growth rate of seedlings	3–2–1	Rapid—Slow
23	Grain length	mm	
24	Grain width	mm	
25	Grain size	mm^2	Grain length × width
26	Grain shape		Grain length/width
27	Apiculus hair length	micron	
28	Empty lemma length	mm	
29	Ligule length	mm	
30	Stoma cell size	micron	Longitudinal size of motor cell
31	Anther length	mm	
32	Pollen grain diameter	micron	
33	Awn length	mm	
34	Premature grain shedding	Index-numbers	Judged visually
35	Alkali-test of endosperm	Index-numbers	2% KOH solution was used.
36	Branch number per panicle		
37	Grain number per unit length		
38	Grain number per panicle		
39	Panicle length	cm	
40	Weight of 100 grains	gm	
41	Germination capacity	%	Hulled grains, 90 days after heading
42	Number of days of germination	Days	

Insular, and the other two were of the *Japonica* or *Temperate-Insular* types.

The characters investigated, 42 in total number, are listed in table 2. As shown in the table, twenty of them have been chosen as the characters usually accounted for in taxonomical descriptions, while the remaining 22 are quantitative ones. The 20 taxonomic (Nos. 1–7 & 9–21) and two quantitative (Nos. 8 & 22) were recorded using code numbers ranging from 1 to 3. For instance, regarding the fringe of the ligule, 1 meant hairless, 3 meant haired, and 2 meant intermediate. If the ligule itself was absent, 0 was given. Strains belonging to one species did not always show the same development of a character. In such cases, the code number occuring with higher frequency was taken as representative for the species. When the number of strains were two, a code number

TABLE 3. *Matrices of correlation coefficients between species*

	Species															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1		.446	.649	.564	.278	.045	−.277	−.040	−.314	−.128	.152	−.137	−.061	−.155	−.172	−.230
2	.367		.403	.204	.356	−.095	−.030	−.105	−.114	−.015	.135	.076	−.157	.147	.073	−.524
3	.530	.676		.396	.171	.170	−.041	.010	−.077	.026	.271	.011	−.183	.048	−.320	−.288
4	.378	.073	.021		.477	.153	−.056	.021	−.129	−.134	.098	−.137	−.005	.101	−.080	−.940
5	.212	.066	.098	.461		−.121	−.286	−.093	−.101	−.131	−.131	.000	−.033	.247	.186	−.248
6	−.239	−.334	−.203	−.254	−.232		.123	.035	.120	.141	.254	.025	−.127	.086	−.284	.205
7	−.293	−.138	−.114	−.051	−.249	.333		.628	.424	.474	.500	.523	.321	−.096	−.085	−.540
8	−.049	−.166	.012	−.032	−.192	.447	.714		.493	.589	.551	.462	.350	.020	−.202	−.039
9	−.168	.087	.089	.155	−.028	.104	.394	.297		.851	.545	.640	.633	.301	.134	−.102
10	.072	.209	.307	.088	−.185	.030	.378	.397	.875		.696	.687	.566	.118	.144	−.326
11	−.040	−.088	.048	.004	−.262	.301	.704	.594	.535	.611		.541	.511	−.067	.074	−.447
12	.004	.127	.054	−.065	.045	.077	.463	.321	.533	.572	.435		.696	.038	.180	−.440
13	−.035	−.034	−.129	.078	−.019	−.206	.133	.522	.651	.514	.373	.453		.150	.286	−.243
14	−.295	−.060	−.026	−.072	−.023	−.031	−.111	−.156	.282	.113	−.214	.059	.222		.301	−.142
15	−.164	.169	−.266	−.104	.244	−.361	−.133	−.001	.159	.075	−.012	.077	.320	.325		−.376
16	−.027	−.167	.109	−.218	−.179	.116	−.404	−.046	−.296	−.304	−.350	−.476	−.160	.005	−.306	

Above the diagonal: Matrix I.
Below the diagonal: Matrix II.

supposedly representative of the species was chosen, after investigating several plants of the strains in question.

For the remaining 20 quantitative characters (Nos. 23–42), measurements were taken using different units (centimeter, micron, etc.), and their species means were standardized taking the general mean for the 16 species to be zero. Further, for these 20 characters, records thus given in standard deviation unit were again transformed into code numbers, taking those over 0.43 to be 3, those between 0.43 and -0.43 to be 2, and those below -0.43 to be 1. These transformed records were used in a part of computations for the sake of comparison between two systems of arrangement, one showing all 42 characters in code number, and the other showing 22 in code number and 20 in standard deviation unit.

Methods of Computation

Correlation matrix. Records for 42 characters in 16 species are given in a 42×16 table. From the figures in the table, correlation coefficients between species were calculated, relating each species in all possible combinations. Two correlation matrices, one based on records for 42 characters given in code number (Correl. matrix I), and the other based on 22 characters in code number and 20 in standard deviation unit (Correl. matrix II), were constructed (table 3). For constructing the latter matrix, correlation coefficients were calculated separately for the two groups of characters, and their arithmetic means were used, assuming that this procedure, although it cannot be justified mathematically, will not seriously interfere with the purpose of combining all information for a survey of the pattern of interspecific variations.

Factor analysis. Several different solutions are known for factor analysis from a correlation matrix. The method used by the writers is the "averoid method," which follows the same procedure as the centroid method except that diagonal elements are excluded from the matrices. From the original correlation matrix (r_{ij}) $(i \neq j)$, loadings of the first factor on the respective species are calculated by the formula:

$$F_i = \sqrt{\frac{n}{(n-1)\sum_i \sum_j r_{ij}}} \cdot \sum_j r_{ij},$$

$$i, j = 1, 2, \cdots n, \quad i \neq j,$$

where n stands for the number of species, and the factor $n/(n-1)$ is required because the means of correlations in the respective columns are used as estimated values of diagonal elements (communality). Then, the matrix $(F_i \cdot F_j)$, which is obtained by multiplying column vector (F_i) by row vector (F_j), is subtracted from the original correlation matrix. The second factor is extracted from the residual matrix by the same formula as used for finding the first factor. This computation is repeated until the factor loadings newly obtained become sufficiently small. In this study, computation was continued until the fourth factor. Then, the values of communality, ΣF^2, were calculated for each species.

In this procedure, if in a row or column there were more minus than plus signs, the signs in the row and column were transformed to the opposite, and after obtaining factor loadings, their signs were readjusted so that the situation before this transformation was restored.

Sokal's weighted variable group method. Details of the procedure of calculation may be referred to Sokal and Michener (1958); a brief explanation of the method will be given below. First, examining the correlation matrix, two species, which show the highest correlation in the row and the column belonging to them respectively, are chosen as the "nucleus" of a group to be formed. Then, the third species which shows the highest average correlation with the members of

TABLE 4. *Factor loadings and communalities obtained for each species*

Species	From cor. matrix I					From cor. matrix II				
	F.I	F.II	F.III	F.IV	h^2	F.I	F.II	F.III	F.IV	h^2
1. *O. sativa*	.176	−.596	.474	−.160	.636	.169	−.627	−.394	.085	.584
2. — *f. spontanea*	.301	−.484	.043	.087	.334	.235	−.449	−.335	.341	.485
3. *O. perennis*	.297	−.477	.153	−.356	.466	.197	−.399	−.240	.206	.298
4. *O. glaberrima*	.393	−.658	−.120	−.271	.675	.197	−.391	−.234	−.043	.248
5. *O. breviligulata*	.174	−.418	.166	.337	.346	.030	−.387	−.338	.227	.316
6. *O. australiensis*	.052	.104	.371	−.292	.236	−.117	.470	.089	−.263	.312
7. *O. latifolia*	.434	.375	−.373	−.456	.676	.503	.402	.090	−.344	.534
8. *O. alta*	.450	.394	.530	−.112	.651	.580	.412	.231	−.251	.623
9. *O. minuta*	.572	.657	.281	.245	.898	.699	.351	.325	.349	.839
10. *O. officinalis*	.686	.560	.234	.007	.839	.780	.206	.061	.233	.709
11. *O. eichingeri*	.746	.262	.210	−.267	.741	.712	.330	.214	−.306	.755
12. *O. malabarensis*	.659	.378	.163	.094	.613	.665	.159	.021	−.051	.471
13. *O. granulata*	.520	.453	.086	.207	.526	.484	.340	.295	.599	.796
14. *O. ridleyi*	.225	−.027	.024	.384	.199	.002	.186	.093	.318	.144
15. *O. brachyantha*	.100	.045	.090	.414	.192	−.003	.268	−.105	.571	.409
16. *O. subulata*	−.763	.369	−.375	.086	.866	−.513	−.127	.035	.263	.350

the nucleus is added to the group, then the fourth and so on. In this procedure, a species to be admitted into a group should have with a member of the group a correlation which is the highest in its own row (or column). The limit to which new members can be admitted is set when the drop of average correlation coefficient by admitting a new species exceeds an empirically determined value (0.20 in the present study).

When the limits are reached in all groups thus formed, correlations between groups are found by the formula (Spearman's sum of variables method)

$$r_{pq} = \frac{\square pq}{\sqrt{p + 2\triangle p}\,\sqrt{q + 2\triangle q}},$$

where $\square pq$ is the sum of all correlations between members of one group with the other group, $\triangle p$ and $\triangle q$ are the sums of all correlations between members of the first and second groups, respectively, and p and q stand for the numbers of members in the respective groups. Then, with the between-group correlation matrix, the same procedure as used for the first grouping is followed. In the present study, the fifth matrix gave a single group of 16 species. The resultant relationships among species are shown in a tree-like diagram, in which the magnitude of correlation between any two joining stems can be read on the abscissa.

RESULTS OF COMPUTATIONS

The correlation matrix based on 42 characters in code number (Correl. matrix I) and that based on 22 characters in code number and 20 characters in standard deviation unit (Correl. matrix II) are given in table 3, above and below the diagonal, respectively. These two matrices appear to be largely comparable with each other. For example, the highest value is found in both of them between *O. officinalis* and *O. minuta*.

1. Results of factor analysis

Loadings of the first to fourth factors and the values of communality (h^2) found for each of the 16 species examined are given in table 4, in which those obtained from correlation matrices I and II are given in the left and right halves of the table, respectively. In several species, the values of communality did not come near to 1.0 enough for assuming that factors contributing to those species were fully extracted. However, extraction of four factors was considered

to be sufficient for the classificatory purpose of this study; theoretically, the species are assumed to be distributed in a multi-dimensional space, which is determined by many axes each representing a factor. Clusters of species could be recognized by examining these four factors as mentioned below.

The data in table 4 show the distribution of species in a four-dimensional

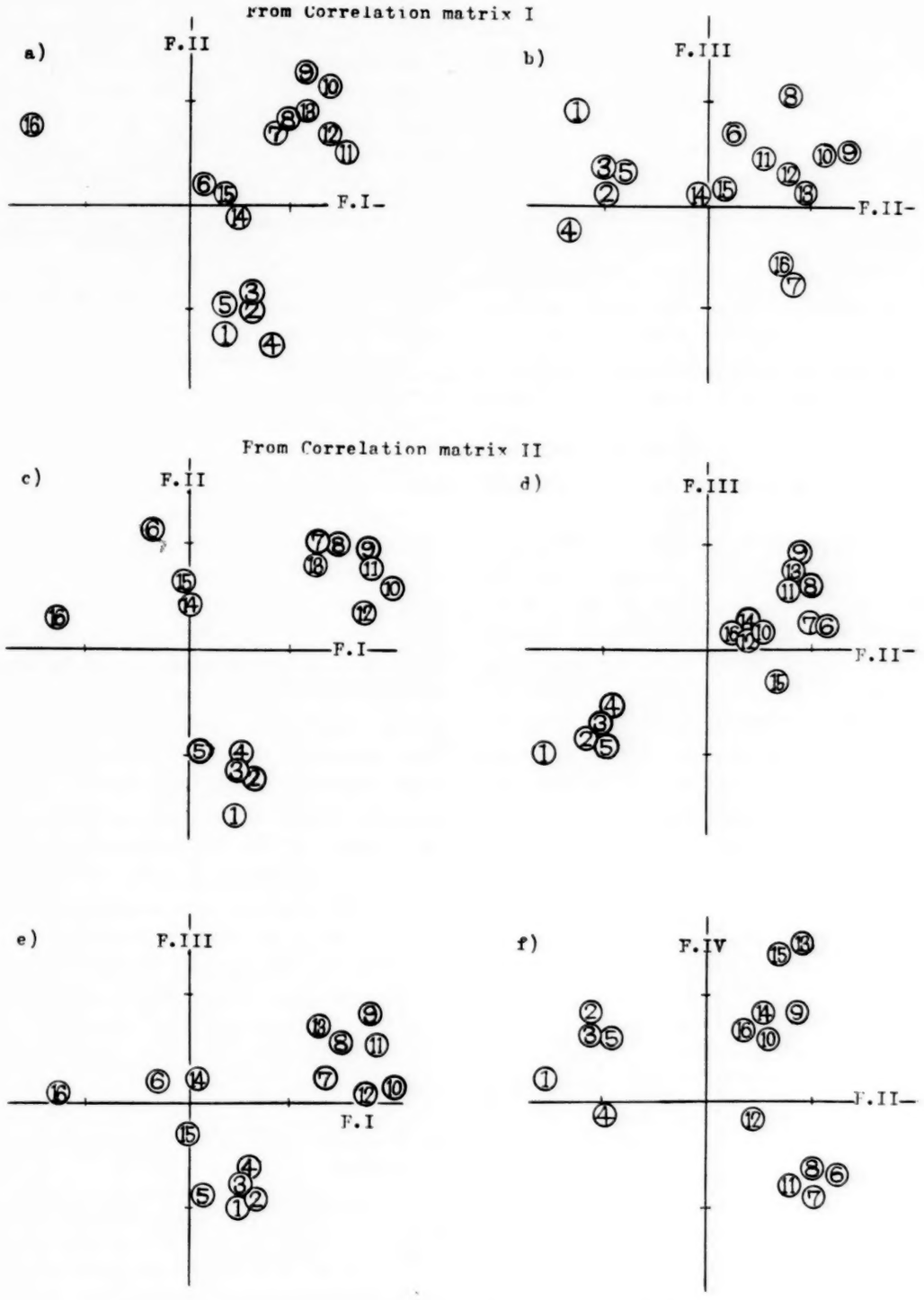

FIG. 1. Distributions of factor loadings on two-dimensional spaces.

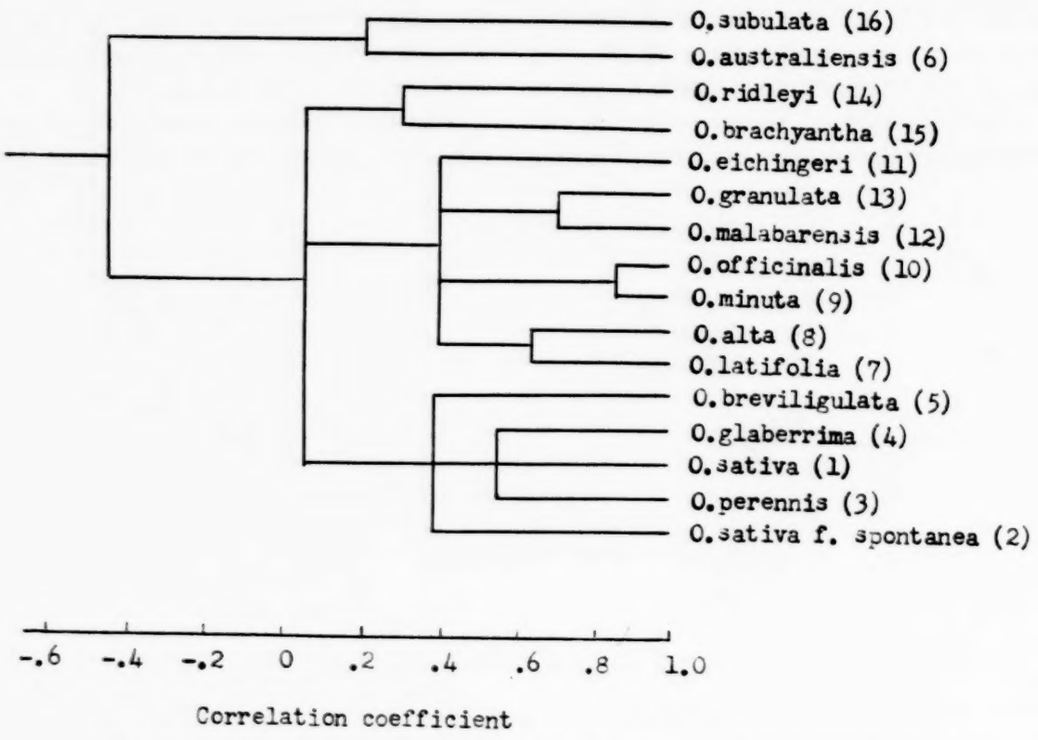

FIG. 2. Diagram of relationships obtained from correlation matrix I.

space. In order to visualize the distribution, it was projected on scattering diagrams by combining two of the four factor loadings in all possible ways. Twelve different distribution patterns (six from each of correlation matrices I and II) were thus obtained. In six of them, all species appeared to be evenly scattered in the diagram. In the other six, the species appeared to form a few clusters. The latter six diagrams (a and b from Correl. matrix I, and c, d, e and f from Correl. matrix II) are set out in fig. 1.

The diagrams in fig. 1 show that there are two main clusters of species, one comprising Species 1, 2, 3, 4 and 5 (*O. sativa*, *O. sativa* f. *spontanea*, *O. perennis*, *O. glaberrima* and *O. breviligulata*, respectively), and the other comprising Species 7, 8, 9, 10, 11, 12 and 13 (*O. latifolia*, *O. alta*, *O. minuta*, *O. officinalis*, *O. eichingeri*, *O. malabarensis* and *O. granulata*, respectively). The remaining four species (6, 14, 15 and 16, or *O. australiensis*, *O. ridleyi*, *O. brachyantha* and *O. subulata*) are scattered outside these clusters.

Accordingly, the 16 species investigated may be classified into three groups. The first group, forming the first cluster, consists of two cultivated species and three wild ones which are known to be closely related with the cultivated species. This group may be called "Sativa group." The second group, consisting of seven wild species forming the second cluster, may be called "Officinalis group," taking the name of its representative diploid member (as shown in table 1, most species in this group are tetraploid). The third is not a group of mutually resembling species; the species scattered outside the former two, which might be distantly related to one another, may be conveniently included, and may be called "miscellaneous group."

2. Tree-like diagrams obtained by Sokal's method

As mentioned already, computation by Sokal's weighted variable group method brings about a tree-like diagram showing interspecific relationships. The diagrams obtained from correlation matrices I and

II are presented in figs. 2 and 3, respectively. Comparing them with each other, and with the results of factor analysis, a consistency is recognized among them. Namely, in both of the tree-like diagrams, the 16 species investigated are divided into two convergent groups and the remaining part, which are just comparable with the three groups found by factor analysis and provisionally named "Sativa," "Officinalis" and "miscellaneous," respectively. It seems that in the genus *Oryza,* there are two relatively distinct groups of species, though several distantly related species are scattered outside of them.

In these tree-like diagrams, the inner structure of the groups, which the factor analysis has not shown, may be read to some extent. However, in the minor structure, the diagrams in figs. 2 and 3 are not completely consistent. An important difference between them is that *O. sativa* is directly related with *O. glaberrima* and *O. perennis* in the former, while with the group of *O. perennis* and *O. sativa* f. *spontanea* in the latter. Another difference is that in the former, *O. australiensis* is linked with *O. subulata,* but in the latter, with the "Officinalis group." It may be difficult to evaluate immediately which is correct, though the diagram in figure 3 seems to be in closer agreement with information from other sources, as discussed later.

Discussion

The methods adopted in this study are worked out upon the following assumptions: (1) The characters observed represent the entire spectrum of interspecific variations under discussion, and (2) no particular weighting is needed for them. These premises have been carefully discussed by Michener and Sokal (1957), and it seems that their views dealing with insect materials can be safely extended to similar investigations in plant materials.

Regarding the first premise, we may

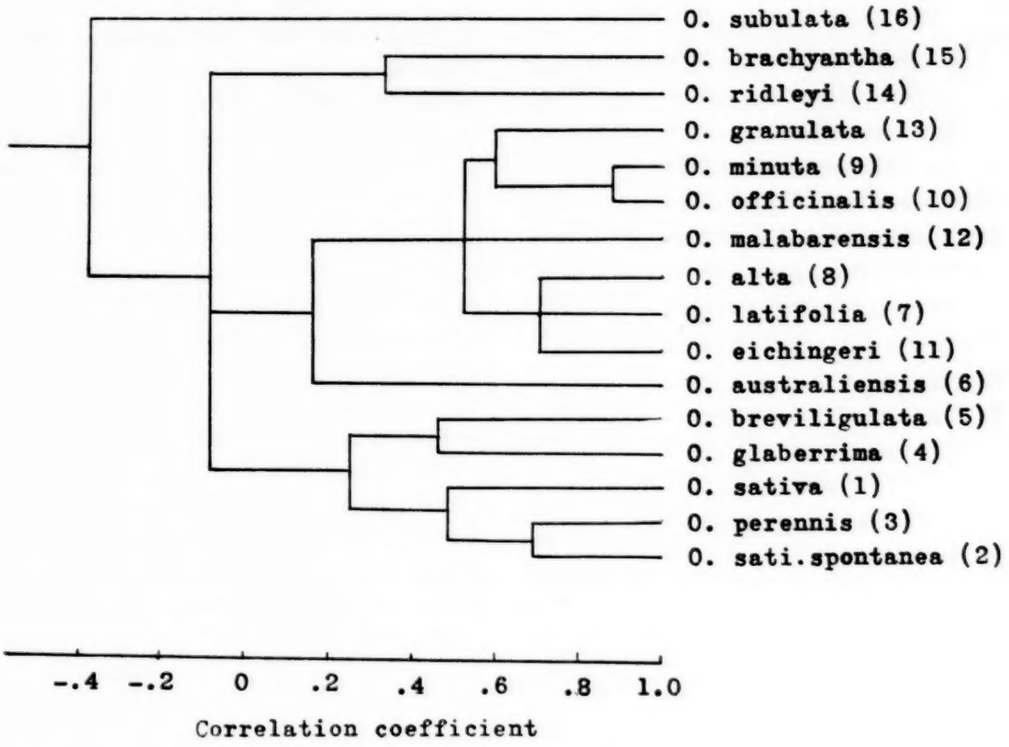

Fig. 3. Diagram of relationships obtained from correlation matrix II (based on 42 characters).

deem that species are differentiated by a large number of genic changes, though we can examine only an easily recognizable part of the results of this process. Then, assuming that both the recognizable and potentially existing differences are similarly the outcome of phylogenetic differentiation, our choice of characters may be regarded as an unbiased sampling from a large population of interspecific differences.

The second premise seems to conflict with the established method of taxonomy, in which weight is given to a few "important" characters. However, the weightings of classical taxonomy seem to be given mostly for the convenience of classification. Theoretically, it may be assumed that the relative importance of characters can be evaluated by the number of relevant genes, or on the basis of their significance in evolutional processes. However, it is difficult to estimate the number of genes controlling a character, because it may differ according to crosses, and in addition to those segregating genes, the genetic background of the species is needed for the expression of characters. It is also difficult to evaluate the significance of a character in evolutional processes. A seemingly minor character can be an indication of underlying physiological processes which are important for the species under a certain condition. Thus, without a bulk of experimental knowledge, it seems practically impossible to evaluate the relative importance of many characters.

Problems remain further regarding possible correlations between the characters. In his classificatory study of human tribes, Rao (1952) tried to eliminate the effect of between-character correlations, by following complicated procedures based on the principle of partial regression. However, correlations between characters are either of developmental or selectional origin. In the former case, they are due to environmental co-fluctuation of characters or pleiotropic effects of genes (environmental and genetic correlations in the ordinary sense). In the latter, correlations are the outcome of phylogenetic differentiation, since certain character combinations might be selected from among numerous ones if selection follows hybridization. In taxonomic studies, therefore, the former type of between-character correlations should preferably be removed, but the latter type should be retained. It is, however, difficult to distinguish between these two types without the aid of specially designed genetic experiments.

The above considerations may lead to the view that no weighting for the characters is better than weighting without sound bases. The number of characters taken in this study, 42, may be said to be the minimum for estimating second degree statistics with a certain reliability. The mathematical techniques employed seem to be rather sensitive to changes in the correlation matrix. However, the results obtained by the two different methods appeared to be comparable. They may then be used as a basis for looking into the phylogenetic relationships of the species.

We should further consider that the species, which had been in their natural habitat in widely different environments, were investigated under experimental conditions. The majority of characters observed may be regarded to be stable, while some might have shown different appearances. It may, however, be said that insofar as we are interested in genotypic variation, the material should be tested under an environment as evenly controlled as possible.

According to Chatterjee (1948), the number of good species known in the genus *Oryza* is 23, though many synonymous names had been used for some of them. As mentioned in the introduction, Roschevicz (1931) considered them to be classified into four sections (table 1). The species examined in the present study cover two-thirds of the known species, belonging to all of the four sections

of Roschevicz. However, of the four sections, Section *Sativae* is the largest one, and the species examined in the present study are for the greater part its members.

Regarding the structure of Roschevicz's Section *Sativae*, an important feature of our study is the demonstration that it contains two relatively distinct groups of species. One of them, called "*Sativa* group," comprises *O. sativa*, *O. sativa* f. *spontanea*, *O. perennis*, *O. glaberrima* and *O. breviligulata*; the other called "Officinalis group," comprises *O. officinalis*, *O. minuta*, *O. eichingeri*, *O. latifolia* and *O. alta*. This grouping agrees well with what Sampath and Rao (1951) have suggested reviewing the results of taxonomical and cytological studies carried out in India.

The "Sativa group" seems, further, to be subdivided into two minor groups, one consisting of *O. sativa*, *O. sativa* f. *spontanea* and *O. perennis*, and the other consisting of *O. glaberrima* and *O. breviligulata*; the F_1s between members of these two groups are highly sterile. This assumption may throw some light on the origin of cultivated rice; taking the tree-like diagram in fig. 3 as a model, the binding link between *O. sativa* f. *spontanea* and *O. perennis* is actually demonstrated by the continuity of variation between these species, which is particularly obvious among strains collected from the delta area of the Godavari River, India (the writers' unpublished data). The links between these species and *O. sativa* are found among the strains collected from Jeypore Tract, Orissa, India, among which many intermediates between wild and cultivated forms occur (Oka, Chang and Narise 1959). According to Portères (1956), the joining link between *O. glaberrima* and *O. breviligulata* is found in Tropical West Africa in wild populations involving these species.

In the "Officinalis group," two pairs of species, *O. officinalis* and *O. minuta*, as well as *O. latifolia* and *O. alta*, appeared to form minor groups. In each of them, the coupled species showed a high resemblance; the only valid distinction between *O. officinalis* and *O. minuta* was that they were diploid and tetraploid respectively.

Looking into these problems, however, we must make the reservation that in the present study, a species was represented by only a few strains which had been identified as belonging to it, and accordingly, intra-specific variations could not be discussed. Further studies in these respects are apparently needed.

O. australiensis, which is according to Roschevicz (1931) a member of Section *Sativae*, appeared in one of the diagrams obtained in our study to be linked with the "Officinalis group," while in the other one to be related to *O. subulata*. In both cases, however, the correlation coefficients were too low to assume their relationships. This species may be considered to be relatively independent. If it is considered to rank as a section, Roschevicz's Section *Sativae* would consist of three sections, *Sativae*, *Officinales* and *Australienses*.

A question also arises regarding *O. granulata*. In the present study, this species appeared to be related to the "Officinalis group." According to Roschevicz (1931), it belongs to a separate section, *Granulatae*, with its sister species, *O. meyeriana*. It is possible that within the range of 42 characters investigated in the present study, variations around this species could not be soundly surveyed.

The link between *O. ridleyi* and *O. brachyantha* found in our study places them in Roschevicz's Section *Coarctatae*. However, the correlation between these two species was too low to consider them to belong to the same group. The results of the present study, thus, seem to suggest that the species outside the "Sativa" and "Officinalis" groups are rather independent of one another. It

seems that, as shown in tree-like diagrams, the major lines of evolutional progress in the genus *Oryza* are directed toward the two groups of species, "Sativa" and "Officinalis," while several species have been left outside the main lines.

Further, as discussed by Michener and Sokal (1957), there arise problems as to how the degree of resemblance between species, as shown by correlation coefficients, serves in the historical sense as an index of phylogenetic distance between them. The answer to this question is difficult if parallel variations occur in isolated groups. Setting such a complication aside, the assumption that the more closely two species resemble each other, the closer is their phylogenetic distance, may be accepted with the reservation that the groups under discussion have had the same rate of evolutional progress. In the materials of the present study, however, the "Sativa group" might have had, with the creation of cultivated forms, a higher rate of evolution than the other groups. Actually, genetically close relationships between the species of the "Sativa group" are shown by the fact that they can be easily crossed and their chromosomes pair in the F_1 almost normally. However, the magnitude of correlations among them was generally lower than that in the "Officinalis group," in which the majority of species are tetraploid and isolating barriers between them might be more profound. This indicates that evolution in the "Sativa group" has been accelerated, as pointed out by Anderson and Stebbins (1954), may be due to the disturbance of habitats by man.

It seems that, for approaching the phylogenetic hierarchy of the genus closer, the results of the present study should be examined in comparison with data from genetic experiments. It may, however, be said that the statistical investigations, as reported in this paper, are useful for obtaining basic informations on which further studies may proceed.

Summary

In order to obtain an insight into the pattern of interspecific variations in the genus *Oryza,* 16 species of the genus were studied by statistical taxonomical methods. A species was represented by one to five strains, and records were taken for each strain regarding 42 characters. Between-species correlations were calculated from the data, and the resultant correlation matrices were analyzed, first with the technique of factor analysis, and secondly by Sokal's weighted variable group method. The diagrams showing interspecific relationships obtained from these computations appeared to be well comparable. It was pointed out that Roschevicz's Section *Sativae* could be divided into distinct groups of species, one represented by *O. sativa* with four other species, and the other represented by *O. officinalis* with six other species. The inner structure of these groups was also shown in tree-like diagrams. Based on interspecific relationships thus estimated, discussion was made about the phylogenetic hierarchy of the genus.

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CHROMOSOMAL POLYMORPHISM IN EGYPTIAN POPULATIONS OF *DROSOPHILA MELANOGASTER*

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Natural populations of many, though not of all, species of Drosophila are polymorphic for the structure of their chromosomes. Most of the naturally occurring chromosomal variations are caused by inversions of sections of chromosomes. The polymorphism often exhibits interesting geographic differences, the populations of some parts of the distribution area of a species being either quantitatively or qualitatively different from other populations with respect to the chromosomal variants which they contain. *Drosophila melanogaster* and *D. simulans* are closely related species the distribution of which is, at present, almost world-wide (the former species being, however, more at home in cooler and the latter in hotter countries). Both species are "animal weeds" associated with man, their original distribution areas being unknown. Nevertheless, the two species contrast sharply in that *D. melanogaster* is very frequently chromosomally polymorphic (Dubinin, Sokolov and Tiniakov 1936, 1945; Bridges and Brehme 1944; Patterson and Stone 1952), while *D. simulans* has not been observed to deviate from a strict chromosomal monomorphism.

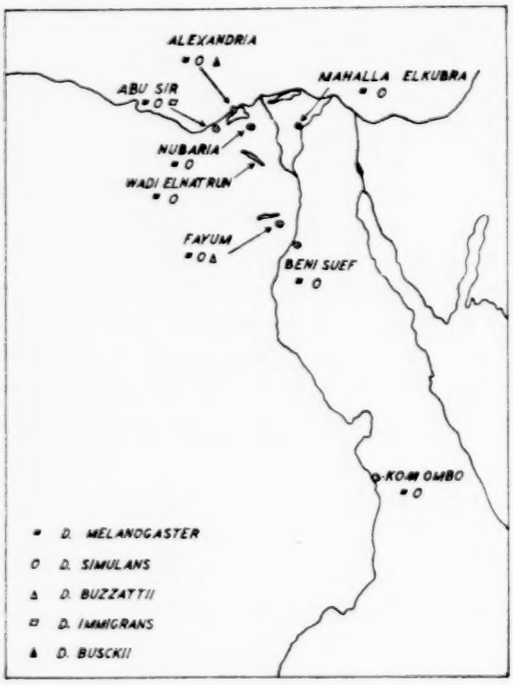

FIG. 1. The localities of collections in Egypt.

The purpose of the present article is to report observations on the chromosomes of certain populations of *D. melanogaster* and *D. simulans* in Egypt. These two species constitute the bulk of the Drosophila fauna of that country, three other species (*D. immigrans*, *D. buzzattii*, and *D. busckii*) having been encountered only sporadically and in few locations.

Material and Methods

Collections have been made in the nine localities shown in figure 1, eight of them being in Egypt and one in Lebanon. Fermenting banana was used as bait, the best time for attracting the flies being before sunset and after sunrise.

The procedure of analysis of the chromosomes consisted in examination of the salivary gland cells of a single F_1 larva obtained in the laboratory from each wild female collected in nature. This method permits the diagnosis of the gene arrangements in two wild chromosomes, contributed respectively by the mother and by the father of the larva examined.

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TABLE 1. *Place and date of capture, and frequency of* Drosophila melanogaster *and* D. simulans

Locality	Date of collection	Flies tested			
		D. melanogaster		*D. simulans*	
Lower Egypt					
Alexandria	26 August 1956	120	206	30	26
	22 October 1956	109	160	20	36
	15 December 1956	22	28	19	26
Abu Sir	1 August 1956	3	1	95	112
Nubaria	7 August 1956	200	248	20	1
	26 September 1957	233	484	29	13
Mahalla El-Kubra	27 July 1957	126	120	50	18
Wadi El-Natrun	9 September 1957	94	49	25	5
Upper Egypt					
Fayum	10 July 1956	75	90	8	3
Beni Suef	21 December 1956	9	1	277	246
Kom Ombo	30 August 1956	3	—	—	—
	5 April 1957	11	10	114	164

Samples of this sort were designated as "egg samples" by Dobzhansky and Levene (1948). The identification of the different gene arrangements was made with the help of the "maps" of the salivary gland chromosomes prepared by Bridges (1935). The numbers of flies tested and the frequencies of the various gene arrangements are given respectively in tables 1 and 2.

THE GENE ARRANGEMENTS IN *Drosophila melanogaster*

In all the populations examined, the prevailing gene arrangement was that described as the standard by Bridges (1935). However, thirteen different inversions have also been recorded, all of them having been seen only in heterozygous condition in the offspring of the wild-collected flies.

The inversions were distributed between the chromosome limbs as follows: four in the left arm of the second chromosome (2L), two in the right arm of the same chromosome (2R), two in the left arm (3L) and five in the right arm (3R) of the third chromosome, none in the X or in the small fourth chromosome. Eight of the thirteen inversions are identical with those previously reported by other workers (see Bridges and Brehme 1944), and five are new. The characteristics and designations of the inversions are as follows:

Chromosome 2, left arm (2L)

1) An inversion with breaks between $23E_2$ and $23E_3$ and between $35F_1$ and $35F_2$. This

TABLE 2. *Frequency percentages of the various gene arrangements found in the second and third chromosomes of* D. melanogaster *flies collected from Egyptian and Safa, Lebanon natural populations*

Locality	Number of larvae examined	2L				2R		3L		3R				
		NS	Cy	M_1	M_2	NS	M	P	M	MO	C	P	K	M
Alexandria	110	8.9	—	1.8	—	2.3	—	—	—	2.3	0.9	—	—	—
Wadi El-Natrun	94	—	23.9	—	—	—	—	4.3	—	1.1	5.3	—	0.5	—
Nubaria	212	—	7.5	—	—	11.8	—	5.2	0.5	0.7	7.5	—	0.2	—
Abu Sir	3	33.3	—	—	—	—	—	—	—	—	16.7	—	—	—
Mahalla El-Kubra	111	—	9.8	—	—	—	—	0.5	—	2.3	—	—	0.5	—
Fayum	54	—	—	—	1.9	—	0.9	—	—	—	—	2.8	—	12.03
Beni Suef	9	—	—	—	—	—	—	16.7	—	—	—	—	—	—
Kom Ombo	11	—	—	—	—	—	—	—	—	—	—	—	—	—
Safa, Lebanon	66	2.3	—	—	—	—	—	—	0.8	—	0.8	0.8	—	—

is the inversion In(2L)NS (Bridges and Brehme, 1944). This inversion was found in Abu Sir, Alexandria, and Safa, Lebanon, with the frequencies 33.3%, 8.18%, and 2.25% respectively. The high frequency encountered at Abu Sir could only be attributed to the very small number of *melanogaster* flies examined from this locality. In(2L)NS was the only second chromosome inversion found in Safa.

2) Another inversion with breaks between $22D_1$ and $22D_2$ and between 33F and $34A_1$ has been designated as In(2L)Cy (Bridges and Brehme, 1944). It was recorded in Wadi El-Natrun, Mahalla El-Kubra, and Nubaria with the frequencies 23.93%, 9.81%, and 7.54% respectively.

3) A new inversion, with the left break before $38F_1$ and the right break following the second band in $40F_1$. Found only in Alexandria with a frequency 1.81%. Designated $In(2L)M_1$.

4) Another new inversion extending from the 21F light band to heavy band 33A inclusive. Designated $In(2L)M_2$; found only in Fayum, with a frequency 1.85%.

Chromosome 2, right arm (2R)

5) An inversion with one break between 52A and $52B_1$ and the other break after 56F. This is In(2L)NS of Bridges and Brehme (1944). Found in Alexandria with a frequency 2.27%, and in Nubaria, 23.58%.

6) A new inversion, the left break being after $54F_1$ and the right break between 58F and 59A. Designated In(2R)M; found only in Fayum with a frequency 0.92%.

Chromosome 3, left arm (3L)

7) An inversion identical with In(3L)P of Bridges and Brehme (1944). Encountered in Beni Suef, Nubaria, Wadi El-Natrun, and Mahalla El-Kubra with the frequencies 16.65%, 5.18%, 4.25%, and 0.45% respectively.

8) A new inversion. It extends from before 66D to 71D inclusive. Designated In(3L)M. Found in Safa and Nubaria with frequencies 0.75% and 0.47%.

Chromosome 3, right arm (3R)

9) The inversion designated In(3R)mo (Bridges and Brehme, 1944). It was found in Alexandria, Mahalla El-Kubra, Wadi El-Natrun and Nubaria with frequencies 2.27%, 2.25%, 1.06%, and 0.7% respectively.

10) This nearly terminal inversion is designated In(3R)C by Bridges and Brehme (1944). Its frequency in Abu Sir was 16.65%, in Nubaria 7.45%, in Wadi El-Natrun 5.31%, in Alexandria 0.9%, and in Safa 0.57%. However, the high frequency in Abu Sir may be attributed to the small number of flies examined.

11) Another inversion with breaks after 89C and after 96A is designated In(3R)P by Bridges and Brehme (1944). Encountered in Fayum and in Safa with frequencies 2.76% and 0.75% respectively.

12) An inversion with breaks before $87A_1$ and before $97A_1$. It seems to be the same as that designated In(3R)K by Bridges and Brehme (1944). Found in Wadi El-Natrun, 0.53%, in Nubaria, 0.23%, and in Mahalla El-Kubra, 0.45%.

13) A new inversion, extending from 86F till after 100D. Designated In(3R)M. Found only in Fayum with a frequency of 12.03%.

Frequency and Distribution of the Inversions in *D. melanogaster*

1—*Alexandria*

Five different inversions were found in this locality, viz. In(2L)NS, $In(2L)M_1$, In(2R)NS, In(3R)Mo, and In(3R)C. Their frequencies are shown in table 2. About 72.72% of the flies in this locality carry the standard gene arrangement. The (2L)NS inversion was the most frequent one. Inversions $(2L)M_1$, (2R)NS, and (3R)Mo were present with nearly equal frequencies, while (3R)C was the least frequent one.

Four cases were found where a larva carried two heterozygous inversions. One of them had (2R)NS and (2L)NS, the second (2R)NS and (3R)Mo, the third $(2L)M_1$ and (2L)NS and the fourth $(2L)M_1$ and (3R)Mo.

Most of the polymorphism in this locality seems to be concentrated in the left arm of the second chromosome.

2—*Wadi El-Natrun*

This locality is a completely isolated valley in the Western Egyptian Desert. Five different inversions were found in this locality, viz. In(2L)Cy, In(3R)C, In(3R)K, In(3R)Mo, and In(3L)P.

Nine larvae were found to be carrying more than one inversion. Three of them had In(2L)Cy and In(3R)C, four carried In(2L)Cy and In(3L)P, one had In(2L)Cy and In(3R)K, and one had In(2L)Cy and In(3R)Mo. Most of the polymorphism was in 2L, while 2R was free from inversions.

3—*Nubaria*

The population of this locality appears to be the most polymorphic population examined. Seven different inversions have been found, viz. In(2R)NS, In(2L)Cy, In(3R)C, In(3R)Mo, In(3R)K, In(3L)P, and In(3L)M.

In all, 31 larvae carried two heterozygous inversions each, and one larva had three different inversions. Six larvae carried (2R)NS

and (3R)C, three had (2R)NS and (2L)Cy, one had (2R)NS and (3L)P, four had (2L)Cy and (3R)C, twelve carried (2L)Cy and (3L)P, one had (2L)Cy and (3R)Mo, one had (2L)Cy and (3L)M, one had (3L)P and (3R)C, one had (3L)M and (3R)Mo, and one had (3L)P and (3R)K. The larva heterozygous for three inversions carried (3L)P, (3R)C and (2L)Cy.

4—*Mahalla El-Kubra*

Four different inversions were found, viz. In(2L)Cy, In(3R)Mo, In(3R)K, and In(3L)P.

5—*Abu Sir*

The Drosophila population from this locality was almost entirely *D. simulans*. Only three *melanogaster* females were caught. Two of them were heterozygous for In(2L)NS, and the third carried In(3R)C.

6—*Fayum*

Four inversions were encountered in this locality, viz. In(2L)M_2, In(2R)M_1, In(3R)M, and In(3R)P.

Most of the polymorphism is concentrated in the third chromosome.

7—*Beni Suef*

The Drosophila population here was mostly *D. simulans*. Nine *melanogaster* females were found, six of them free from inversions, while the remaining three carried In(3L)P.

8—*Kom Ombo*

Eleven *melanogaster* females were caught, and they were free from any polymorphism.

9—*Safa, Lebanon*

Four inversions were encountered in this locality, viz. In(2L)NS, In(3L)M, In(3R)P, and In(3R)C.

Lack of Inversions in *D. simulans* Populations

The populations of *D. simulans* from the nine localities listed in table 1 were examined cytologically. In sharp contrast to *D. melanogaster* from the same localities, all the populations of *D. simulans* were found to be free from any perceptible chromosomal polymorphism.

Natural Hybrids between *D. melanogaster* and *D. simulans*

Despite their external phenotypic similarity, *D. melanogaster* and *D. simulans* show a complete reproductive isolation. They cross only with difficulty, one of the sexes in the hybrid progenies is inviable, and the viable sex is absolutely sterile. Nevertheless, two of the larvae examined cytologically proved to be hybrids between the two species. One of them came from a female collected at Abu Sir and the other at Kom Ombo. It is evident that these females were insemenated in their natural habitats by males of foreign species.

Conclusions and Summary

The two commonest Drosophila species in Egypt, namely *D. melanogaster* and *D. simulans* contrast strikingly in that the populations of the former species exhibit considerable chromosomal polymorphism, while those of the latter species are chromosomally monomorphic.

The populations of *D. melanogaster* found in different localities in Egypt may differ both in the amount and in the quality of the polymorphism present in them. In all, thirteen different inversions have been encountered, eight of which proved to be identical with inversions recorded by various investigators in other countries, and five proved hitherto unknown ones. None of the inversions were recorded in the populations of all the localities examined, although some of them are quite frequent in some localities. The standard gene arrangement is, however, the prevailing one in all chromosomes and in all localities. No individual homozygous for any of the inversions has been encountered. This is unlikely to be due entirely to the inadequate size of the samples examined; low adaptive values of the inversion homozygotes offer a more probable explanation.

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THE ECOLOGICAL SIGNIFICANCE OF CORRELATION PLEIADES [1]

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The concept of correlation pleiades was originally advanced by Terentjev (1931). It is based on the presence of correlations between some quantitative characteristics, e.g., between dimensions of certain parts of an organism (or between different measurements within one part), and, at the same time, on the absence of correlations between these and other parts. The existence of correlation pleiades leads to theoretically important conclusions.

The presence of correlations between, for instance, the dimensions of some parts of an organism and the absence of correlations between the dimensions of these and the other parts of the same organism indicate the independence of certain developmental processes with respect to other processes within the organism. The pleiades are thus a manifestation of differentiation.

With respect to a given part, other parts of the same organism basically constitute its environment. The origin of correlation pleiades in the course of evolution is an indication of the increasing independence of certain developmental processes with respect to environmental factors, including the influences exerted by the other parts of the same organism. The study of correlations makes it possible to establish the degree of independence reached. The adoption of such a quantitative criterion provides an adequate basis for the introduction of the comparative method in the study of the process by which this independence is attained. Investigation of correlation pleiades has a considerable practical importance in science, as well as in agriculture and medicine.

The application of statistical methods to the solution of some taxonomical problems depends entirely on the success in the choice of non-correlated characters (Heincke, Fisher; see Lubishchev, 1959, and Terentjev, 1959). The general theory of the correlation pleiades, including their ecological interpretation, provides the pattern for selection of the non-correlated characters or "indicator-characters," as Terentjev has termed them.

In agricultural practice, especially in the industrial processing of the products of plant and animal husbandry, ever increasing practical importance is played by the standardization of raw agricultural materials. But a standard, or norm, is merely the expression of autonomy of characters, i.e., of their independence from the fluctuations of environmental factors during the development of these characters. The principle of correlation pleiades brings us close to the controlling of the process of standardization. To know how pleiades arise, is to know the mechanism of the origin of independence from environmental variability.

The general theory of correlation pleiades must obviously answer such questions as why correlation pleiades exist, and why it is a given part and not another one that escapes from the network of correlations within the organism, and either retains constant quantitative characteristics or varies according to its own principles more or less independently from the variation of other parts?

The general theory of correlation pleiades can be formulated only if some

[1] Delivered at the Leningrad State University Conference on Quantitative Biology, May 1958 (see Berg, 1959b).

general principle were established, which would make it possible to consider any instance of presence or absence of the pleiades as some particular manifestation of that principle. The establishment of such a principle would enable us to predict the existence of correlation pleiades. It is obvious, however, that this task has to be confined in each specific instance to the particular branch of science involved. Each branch of biology is capable of solving problems concerning living organisms, but the answer will always be restricted to those aspects of the object that this particular branch is investigating. However, any principle, expressed in terms of a strictly defined branch of science, would, nevertheless, be a general principle, since it will allow the discovery of all the features common to the diverse elements of the phenomenon considered from a certain viewpoint.

The purpose of the present paper is to give an ecological interpretation of the phenomenon of correlation pleiades extending the previous report on this subject (Berg, 1959b). Consequently, the focus of attention in the discussion is provided by the principle of adaptation.

There are two aspects to this problem. Firstly, the adaptive significance of the correlation pleiades must be established. For this, the variability of the very phenomenon of correlation pleiades must be investigated to start with. That is to say that it is necessary to find instances of presence as well as of absence of correlation pleiades, or that a difference in the degree of correlation between analogous characters in different species

TABLE 1. *Ecological and morphological description of the plants studied*

Species	Number of plants studied	Mode of pollen transfer	Type of floral symmetry	Position of the flower on the plant	Presence or absence of tubular parts
1. *Triticum aestivum*	100	self-pollinated	dorsiventral	lateral	lacking
2. *Elymus arenarius*	50	wind-pollinated			
3. *Hordeum vulgare* var. *pallidum*	100	self-pollinated			
4. *H. vulgare*, var. *nudum* (hulless)	100				
5. *Anemone nemorosa*	65	pollinated by unspecialized insects	radial	terminal	
6. *Fragaria collina*	50				
7. *Papaver* sp.	45				
8. *Cosmos bipinnata* (inflorescence)	150				
9. *Lychnis viscaria**	40	pollinated by butterflies	radial	lateral and terminal	present
10. *L. chalcedonica**	30				
11. *Melandrium silvestre**	50				
12. *Nicotiana alata*	40		dorsiventral	lateral	
13. *Phlox divaricata*†	50			lateral and terminal	
14. *Geranium pratense*	65	pollinated by bees and bumblebees	radial structure functioning as dorsiventral as the result of lateral position	lateral	lacking
15. *Chamaenerium angustifolium*	50				
16. *Linaria vulgaris*	30		dorsiventral		present
17. *Delphinium elatum*	53				
18. *Digitalis ambigua*	75				
19. *D. purpurea*	50				
20. *Tropaeolum majus*	150				

* In *L. viscaria*, *L. chalcedonica* and *M. silvestre* the false tube of the corolla is formed by laminar petals. The calyx is tubular.

† In *P. divaricata* the flower is bilateral in consequence of the position of stamen.

TABLE 2. *The measurements studied and the symbols used for them in tables 3–7*

Vegetative parts		Stem	Height	A
		Leaf	Length	B
			Width	C
			Number	D
		Inflorescence	Length	F
			Number of florets (or of spikelets)	G
Reproductive parts	Perianth	Corolla	Flower diameter	I
			Petal length (length of flowering glume)	J
			Length of tubular parts	K
		Calyx	Sepal length (glume length)	M
			Calyx length	N
		Flower	Stamen length	O
			Pistil length	P
		Grain	Grain length	L

must be established. Next, it should be determined what complex of ecological relationships is associated with the presence or absence of a particular pleiad. In the course of such investigation, the adaptive significance of the presence or absence of a correlation pleiad should be revealed and the selective factor responsible for the origin of this pleiad identified. The second aspect, that dealing with the general principle of the origin of correlation pleiades, can be attacked only after this operation is applied to at least one specific case. This general principle may be sought from comparisons of mechanisms of initiation of a variety of pleiades, for the purpose of identifying features common to all of them.

MATERIAL AND METHODS

Nineteen species of herbaceous plants have been studied in order to determine the variability in the degree of dependence between the dimensions of different parts of an organism. One of the species studied, viz. barley (*Hordeum vulgare*) is represented in our study by two varieties: *pallidum*, Pushkinsky ozimyi, no. 1, and the hulless variety *nudum*. The wheat used in this study, *Triticum aestivum* (variety *diamant*) was studied both by Krasnovidova in 1956 and by the present author in 1957. Only the results of the second series of investigations are given in this paper. The list of the species investigated as well as the number of the specimens studied for each species is given in table 1. The list of characteristics of plants and a key to the letter symbols denoting them in the following tables are given in table 2. Not all the species were studied with respect to all characters listed, the range of the number studied for a species being from three to ten.

The plant parts investigated were classified into vegetative and reproductive according to the specific significance of form and dimensions for the process of reproduction. Stems, leaves and inflorescences are vegetative parts; stamen, pistil, corolla and calyx are reproductive parts. All the organs, the shape and dimensions of which are important in determining the success or failure of pollen transfer are thus denoted as reproductive. Hence, the inflorescence of *Cosmos bipinnata*, analogous in its function to radial flowers is regarded as

TABLE 3. *Correlation coefficients between the dimensions of vegetative and reproductive parts of* Chamaenerium angustifolium *Scop.*

	A	B	C	D	F	G	I	M	O
B	0.82 ±0.05								
C	0.70 ±0.07	0.84 ±0.04							
D	0.78 ±0.05	0.66 ±0.08	0.41 ±0.12						
F	0.85 ±0.04	0.77 ±0.06	0.74 ±0.06	0.55 ±0.10					
G	0.89 ±0.03	0.90 ±0.03	0.69 ±0.07	0.56 ±0.10	0.88 ±0.03				
I	0.05 ±0.14	0.20 ±0.14	0.44 ±0.11	−0.03 ±0.14	0.16 ±0.14	0.07 ±0.14			
M	0.09 ±0.14	0.24 ±0.13	0.39 ±0.12	−0.10 ±0.14	0.13 ±0.14	0.08 ±0.14	0.70 ±0.07		
O	−0.09 ±0.14	0.08 ±0.14	0.24 ±0.13	−0.22 ±0.13	−0.13 ±0.14	−0.12 ±0.14	0.50 ±0.11	0.49 ±0.11	
P	0.32 ±0.13	0.48 ±0.11	0.47 ±0.11	0.08 ±0.14	0.29 ±0.13	0.29 ±0.13	0.57 ±0.09	0.50 ±0.11	0.44 ±0.11

a reproductive part, while the inflorescences of all the other species studied are classified as vegetative parts. The outer parts of the perianth of different species (sepal, empty glume), as well as the inner parts (petal, flowering glume) are respectively denoted by the same symbols. Analogy rather than homology was deemed to be of significance. The resemblance in function of the outer parts of the perianth of different plants justified the search for pleiades involving their dimensions. This also holds for inner parts. Minor differences in functioning of parts justify the hope that the reasons for the presence or absence of a correlation pleiad could be understood and that an ecological interpretation of pleiades may be obtained.

The dimensions of different parts were in all cases measured and analyzed within the same group of specimens. Correlations as expressed by correlation coefficients were computed for the dimensions of all parts studied within each species.

It may be seen from table 1 that the diversity of the material involved in this study afforded the possibility of comparison of plants widely differing in both their ecological characters (e.g., the mode of pollen transfer) and their morphology (the structure and position of flowers as well as the type of their symmetry). Among the plants studied there are self-pollinators (*T. aestivum, H. vulgare*), anemophiles (*E. arenarius*), entomophiles having no specific insect pollinators (*A. nemorosa, F. collina, Papaver* sp., *C. bipinnata*), entomophiles pollinated in their native habitats either now or in the past by butterflies (*L. viscaria, L. chalcedonica, M. silvestre, N. alata, Ph. divaricata*) and, finally, plants having such specialized pollinators as bees and bumble-bees (*G. pratense, Ch. angustifolium, L. vulgaris, D. elatum, D. ambigua, D. purpurea* and *T. majus*). Our material includes plants with flowers having tubular parts (*L. viscaria, L. chalcedonica, L. vulgaris, D. elatum, Ph. divaricata, T. majus*), and plants with flowers completely transformed into a tube (*D. ambigua, D. purpurea*), as well as plants whose flowers are devoid of tubular parts (*G. pratense, Ch. angustifolium*), all of them possessing specialized pollinators. Plant species selected for this study vary also with respect to

TABLE 4. *Correlation coefficients between the dimensions of vegetative and reproductive parts in* Delphinium elatum *L.*

	A	B	G	H	K
B	0.53 ± 0.10				
G	0.67 ± 0.08	0.59 ± 0.09			
H	0.45 ± 0.11	0.57 ± 0.09	0.49 ± 0.10		
K	0.22 ± 0.13	− 0.10 ± 0.14	0.12 ± 0.14	− 0.03 ± 0.14	
M	0.16 ± 0.13	− 0.10 ± 0.14	− 0.13 ± 0.14	− 0.08 ± 0.14	0.40 ± 0.12

TABLE 5. *Correlation coefficients between the dimensions of vegetative and reproductive parts in* Triticum aestivum, *variety* diamant

	A*	F	G	M	I_1†	I_2†
F	0.73 ± 0.05					
G	0.61 ± 0.06	0.79 ± 0.04				
M	0.60 ± 0.06	0.69 ± 0.05	0.51 ± 0.07			
I_1	0.57 ± 0.07	0.70 ± 0.05	0.54 ± 0.07	0.69 ± 0.05		
I_2	0.67 ± 0.06	0.69 ± 0.05	0.59 ± 0.07	0.71 ± 0.05	0.87 ± 0.02	
L	0.43 ± 0.08	0.45 ± 0.08	0.36 ± 0.09	0.47 ± 0.08	0.61 ± 0.06	0.58 ± 0.07

* Length of the upper internode.
† I_1—length of lemma; I_2—length of palea.

the degree of oligomerization and fixation of the number of parts of the flower and the perianth.

RESULTS

It has been established that, among the nineteen species studied, twelve species have correlation pleiades, involving dimensions of some parts investigated (*L. viscaria, L. chalcedonica, M. silvestre, N. alata, Ph. divaricata, G. pratense, Ch. angustifolium, L. vulgaris, D. elatum, D. ambigua, D. purpurea* and *T. majus*), while seven species have no correlation pleiades with respect to the dimensions of the analogous parts (*T. aestivum,* both varieties of *H. vulgare, E. arenarius, A. nemorosa, F. collina, Papaver* sp., and *C. bipinnata*). In the former twelve species the parts studied form two groups, within each of which all the dimensions are positively correlated with each other, while bearing no or very little correlation with the dimensions recorded in the other group. Such grouping of characters indicates, following Terentjev, presence of correlation pleiades.

In the latter seven species no correlation pleiades were observed. The dimensions of all the parts investigated were closely intercorrelated, thus forming, as it were, a single correlation pleiad. Such a phenomenon is regarded as indicating absence of correlation pleiades, since only the absence of correlations between the dimensions of certain groups of characters delimits the pleiades and thus makes it possible to distinguish between the presence of correlation pleiades and the mere existence of correlations.

Only representative data are given in the present article. The species with correlation pleiades are represented by *Chamaenerium angustifolium* (table 3) and *Delphinium elatum* (table 4), while

TABLE 6. *Correlation coefficients between the dimensions of vegetative and reproductive parts in* Hordeum vulgare, *var.* pallidum, *Pushkinsky ozimyi, No. 1*

	A*	B	F	G	M	I_1†	I_2†
B	0.65 ± 0.06						
F	0.38 ± 0.09	0.56 ± 0.07					
G	0.37 ± 0.09	0.58 ± 0.06	0.93 ± 0.01				
M	0.46 ± 0.08	0.54 ± 0.07	0.68 ± 0.05	0.66 ± 0.06			
I_1	0.38 ± 0.09	0.43 ± 0.08	0.60 ± 0.06	0.58 ± 0.06	0.52 ± 0.07		
I_2	0.28 ± 0.09	0.44 ± 0.08	0.66 ± 0.06	0.66 ± 0.06	0.66 ± 0.06	0.57 ± 0.07	
L	0.34 ± 0.09	0.47 ± 0.08	0.59 ± 0.06	0.62 ± 0.06	0.50 ± 0.08	0.52 ± 0.07	0.58 ± 0.06

* Length of the upper internode.
† I_1—length of lemma; I_2—length of palea.

TABLE 7. *Correlation coefficients between the dimensions of vegetative and reproductive parts in* Anemone nemorosa *and* Geranium pratense

Species		A	B
Anemone nemorosa	B	0.75±0.05	
	J	0.79±0.05	0.77±0.05
Geranium pratense	B	0.71±0.06	
	I	0.01±0.13	−0.05±0.13

the species having no pleiades are exemplified by *Triticum aestivum* (table 5) and *Hordeum vulgare* (table 6). Correlation coefficients between the dimensions of all the parts studied are shown for these four species. Correlations between the dimensions of vegetative and reproductive organs are indicated within the frame, shown on the table; those between the dimensions of different vegetative parts are shown above the frame, and correlations between the dimensions of different reproductive parts are to the right of the frame. As it may be seen from tables 3 and 4, in both *Ch. angustifolium* and *D. elatum* the dimensions of vegetative organs are closely correlated with one another, as are the dimensions of reproductive organs, but the correlation between dimensions of vegetative organs on the one hand and those of reproductive organs on the other hand is low or, in fact, in most cases, there is no correlation. As shown in tables 5 and 6 in wheat and barley all the dimensions studied are correlated. Correlation coefficients between the dimensions of grains (denoted by L) and those of other parts of the organism are somewhat smaller than all other correlation coefficients. This might well be expected, the grain being essentially an organism of the next generation with a genotype of its own, and not a part of the parent organism. In wheat and in barley there are no correlation pleiades with respect to the characters studied.

As shown by table 7, in *Geranium pratense* the size of the corolla is not correlated with the dimensions of the stem and leaf, whereas in *Anemone nemorosa* such a correlation does exist.

Thus, variation, or the existence of correlation pleiades, has been established. In all instances in which pleiades were present, it was the flower that had become independent of the correlations within the plant organism.

DISCUSSION

The next step of the analysis deals with the determination of the complex of associated traits which governs the presence or absence of correlation pleiades. As shown in table 1, neither floral symmetry of the position of the flower on the plant have a bearing on whether pleiades are present or absent. But the method of pollen transfer is indeed linked with this factor. All plants with specific insect pollinators, be they butterflies or bees, have correlation pleiades. All plants lacking specific pollinators, be they self-pollinated, anemophiles or entomophiles without specialized insect pollinators, lack correlation pleiades. Presence of correlation pleiades involving the characters studied indicates a certain degree of independence of the flower dimensions. The adaptive significance of this autonomy is perfectly evident. The dimensions of the flowers of plants having specific insect pollinators are not only independent from the dimensions of the plant, but also, as it has been shown by our previous investigations (Berg, 1956, 1958), they are more stable than the dimensions of stems, leaves and inflorescences. Such stability of dimensions of zygomorphic flowers and their independence from the variation of environmental conditions including organ interactions are the indispensable conditions of successful pollen transfer. All this refers in the first place to the dimensions of stamen filaments and of the tubular parts of flowers.

Some parts of the flower act as distance and orienting signals for the pollinating insect (laminal parts of the perianth), others help to deposit the pol-

len on some definite part of the pollinating insect's body (the tubular parts of the perianth and the stamen filaments). The strict localization of the deposition of the pollen on some definite part of the body of a specialized pollinating insect was already discovered by Darwin (1862) in *Orchidaceae*. Recently Shvanvich (1952, 1956) also observed it and studied it in detail in *Papilionaceae*. The structure of some flowers, the pollen of which is transferred by specialized pollinating insects, is adapted for the precise deposition of the pollen on a definite part of the body of the pollinating insect.

Our investigation shows the remarkable stability and independence of the parts of the flowers specialized to deposit the pollen on a definite point of the body of the pollinator. The biological function of the adaptations to localize the pollen deposit on some definite part of the body of the pollinating insect is probably to reduce the wastage of the pollen. This is especially true if the pollen is deposited in a location of the insect's body which is out of reach of its pollen-removing organs. Now, the precise localization of the pollen deposit is functionally effective only if the flowers of the species have all approximately equal dimensions. The size of every flower must, thus, be adapted not only to the dimensions of the specialized insect pollinators, but the flowers of different individuals in a cross-pollinating population must also be rather uniform. A flower which locates its pollen on the insect's body in a wrong way is likely to have no progeny. The existence of correlation pleiades with respect to the characters studied is thus inevitably concomitant to the adaptations to localize the pollen deposit on some definite part of the body of the pollinating insect. The adaptations to localize the pollen deposit involve: the acquisition in the process of evolution of zygomorphic flower structure reducing to the minimum the number of possible positions on the flower of such a bilaterally symmetrical creature as an insect pollinator; the development of tubular parts, reducing the arena where the critical events take place; the reduction in number (oligomerization) and the rigid fixation of the number of homologous parts; and, finally, lateral position of flowers on the plant, which even when radially-symmetrical, function as if bilaterally-symmetrical. Each of the twelve species possessing specific insect pollinators has at least one of these evolutionary adaptations (see table 1). The presence of correlation pleiades is thus associated with the mode of pollen transfer, involving specific insect pollinators, with adaptations to localize the pollen deposit on some definite part of the body of the pollinating insect, and with, at least, one of the structural features, providing for localization. The absence of correlation pleiades is associated with absence of specific insect pollinators, as well as with absence of structural features providing for pollen localization. In this manner the nature of the complexes of regularly interdependent characters which involve presence or absence of correlation pleiades can be said to have been established.

General Conclusions

What is then the mechanism of selection, which leads to correlation pleiades involving reproductive and vegetative organs in entomophilous plants pollinated by a limited number of specialized insect species? In this particular instance this mechanism is perfectly obvious. The advantage of the independence of dimensions of reproductive organs as well as the disadvantage of the dependent type of their development are self-evident, the plants with dependent flowers being eliminated, since their pollen could not be delivered to the stigma of another flower, while the plants exhibiting independence had a much greater chance to leave progeny. Pollinating insects were, then, selective agents for the dimensions of the floral parts, and for their standardization, which removed these

parts from the developmental interrelations and created independent correlation pleiades for reproductive organs, as well as for vegetative parts of the same organism. Standardizing selection of dimensions can be accomplished only by insect species that have themselves uniform dimensions, i.e., bees and bumblebees whose development takes place in cells of standard size. Pollinating insects take no part whatever in the formation of flowers in the course of development. Their function is only to test fitness. The discrepancy between the factors participating in the formation of the character and the selective forces determining its function is apparently a general ecological principle of making the dimensions of some parts independent of those of others, of some traits from others, that is, the general principle of the origin of correlation pleiades.

In this instance, the accomplishment of a certain function requires the precise correspondence of dimensions in organisms incapable of exerting any direct influence upon each other during their ontogeny. Such a situation is by no means unique. The biotic components of the environment taking no part whatever in the development of the individuals of a certain species frequently are of decisive significance as selective agents within the species. This is just the case in the interrelation between predators and prey, males and females, the animals dispersing the seeds of plants and such plants themselves. It is the characters and the organs accomplishing the function of communication with the biotic components of the environment that acquired independence from random environmental variations and form correlation pleiades. These organs and characters, namely, flowers, fruit and seed coats and leaves involved in the process of reproduction in plants, the nervous system, sex organs, the digestive system, behavior associated with reproduction and social instincts in animals, and so forth, are the vast field, where the processes of increased autonomy are likely to take place and where, consequently, correlation pleiades are to be sought. In all these instances the general principle underlying the origin of correlation pleiades is the discrepancy between the agencies participating in the formation of the character and the selective forces determining its function.

As it has been shown in the frog, *Rana ridibunda* Pall., by Terentjev (1931), the dimensions of sense organs and of organs participating in reproduction are characterized by the least dependence on the dimensions of the other parts of the organism. Bertalanffy and Pirozynski (1952) discovered the disproportionality of growth of brain and liver in rat embryos. The allometric growth of just these organs is in agreement with our hypothesis.

The autonomy of vital processes of a given species is based on homeostatic mechanisms of cytological, developmental (ontogenetic) and populational levels. The significance of developmental and genetic homeostasis has been recently emphasized by Lerner (1954). The genotype as a whole is a homeostatic mechanism, acting on the cellular, developmental and population levels of organization of the species. The hereditary factors play the role of stabilizers, being present in the cells of the developing organism. The origin of autonomy in the course of evolution is accompanied by the increase in the relative importance of internal factors of development, as compared to the external or environmental factors (Kamshilov, 1939, 1946; Schmalhausen, 1938, 1939, 1946). It should be clear that the dimensions of an autonomous flower are controlled by some factors, internally located, most probably by means of some intracellular mechanism.

In our example, the increased significance of internal developmental factors is the result of the distinctness of the formative and the selective factors. In higher organisms formative and selective

factors rarely coincide. Suffice it to point at the alternation of generations. The formation of an organism proceeds under one set of external conditions; its life under another. Parents engender offspring which are to live in conditions which cannot be identical with those in which they existed themselves, let alone, in conditions of their early ontogeny. Only in the course of ontogeny does the progeny begin to assume a resemblance to the parents. The organism thus does not inherit from its parents its traits and characteristics but simply an arrangement which contains in coded form a program for development. The totality of hereditary potentialities ensures a certain autonomy of the vital processes vis-a-vis the factors of the environment. The programming of development is of significance only in forms which have alternation of generations, individual development or both. The whole sense of the hereditary code lies in the discrepancy between the formative and the selective factors. This discrepancy is thus responsible for the initiation of all kinds of autonomy and of all the mechanisms ensuring independence, including the origin of the coding itself.

What then is the practical significance of the general ecological principle underlying the origin of correlation pleiades? The answer is that this principle gives us assurance that the selection of stabilized states of any character, the kind of selection that has been accomplished by insect pollinators with respect to flower dimensions, can be an effective means for breaking undesirable correlations and therefore for the creation of the required stabilized state of a given character independent from the fluctuations of environmental factors.

Summary

The phenomenon of correlation pleiades, i.e., the presence of correlations between some quantitative characteristics of certain parts of an organism and at the same time by the absence of correlations between these and the other parts demonstrates the independence of certain developmental processes with respect to other processes within the organism. The study of correlation sets makes it possible to establish the degree of such independence. The adoption of a quantitative criterion provides an adequate basis for the comparative method in the study of stabilizing selection.

Nineteen species of herbaceous plants have been studied in order to determine the variability in the degree of dependence between the dimensions of different parts of an organism. It has been established that twelve species have correlation pleiades, while seven species have no correlation pleiades with respect to the dimensions of analogous parts. In all the instances of existence of pleiades it was the flower that had become free of the correlation interdependences within the plant organism. Existence of correlation pleiades is associated with the mode of pollen transfer, involving the presence of specific insect pollinators, with adaptations to localize the pollen deposit on some definite part of the body of the pollinating insect, and with at least one of the structural features providing for such localization (zygomorphism, presence of tubular parts, lateral position of flowers, oligomerization and a rigid fixation of the number of homologous parts of a flower).

Insects were the selective factor stabilizing the dimensions of those parts of flowers which participate in the process of the precise localization of pollen on the insect body. Stabilizing selection of flower dimensions created correlation pleiades for reproductive organs, as well as for vegetative parts of the same organism. The general principle underlying the origin of developmental homeostasis in the process of evolution is the discrepancy between the agencies participating in the formation of the character and the selective forces determining its function. The same principle is responsible for the origin of correlation pleiades.

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EVOLUTIONARY CONSEQUENCES OF HYBRID STERILITY AND VIGOR IN TOADS[1]

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Introduction

Hybridization between the Gulf Coast toad, *Bufo valliceps* Wiegmann, and Fowler's toad, *Bufo fowleri* Hinckley, is unproductive (Volpe, 1956). F_1 hybrids derived from the cross, *valliceps* ♀ × *fowleri* ♂, cytolyse in the gastrula stage of embryonic development. In contradistinction, the majority of the hybrids from the reciprocal cross, *fowleri* ♀ × *valliceps* ♂, are viable, but all are sterile and of only one sex (male). The sterility of the adult hybrid is due to the rudimentary condition of the testes; spermatogonia are present but spermatocytes are invariably lacking.

Since the interspecific hybrids are either inviable or sterile males, one might assume that the evolutionary effects are insignificant. However, a relatively large number of natural hybrids are found in localities in eastern Louisiana where the ranges of the two species overlap (Volpe, 1956, 1957). There is no evidence that the reproductively ill-adapted male hybrids are subject to adverse selection. The testicular irregularities of the hybrids stand in sharp contrast to the normality of the soma. Indeed, as shown in the present report, the hybrids appear to be equal, if not superior, in somatic vigor to individuals of either parental species.

The hybrids, although sterile, are active participants in mixed breeding congregations. They engage in amplexus with females of each species, although more often with *fowleri* females, and are capable of causing the females to extrude their eggs. Evidently, the misclasped females' contribution to the gene pool of the next generation is nil. Data are presented herein which suggest that the hybrids are affecting significantly, although not necessarily in a detrimental way, the genetic variability of one of the recurrent species, *fowleri*.

Background of the Problem

It is not uncommon to encounter mixed breeding aggregations of *B. valliceps* and *B. fowleri* in the coastal plains of Louisiana, where the two species are sympatric. Orton (1951) and Liner (1954) have observed interspecific matings at different localities in southeastern Louisiana. Mis-mated pairs have been noted by the writer at a local site, Audubon Park, adjacent to the Tulane University campus. The water table in the open and grassy portions of the park remains near the soil surface throughout the year. The soft wet surface is inundated by heavy rains. In the northern part of the park, large congregations of toads occur periodically in shallow, rain-flooded ditches, lined by grasses and thickened by dense tufts of sedges and rushes.

The accessibility of the natural hybrid population facilitated a combined field and laboratory study, initiated in 1954. Laboratory crosses were performed to ascertain the extent to which interspecific hybridization is possible (Volpe, 1956). As stated in the introduction, the only

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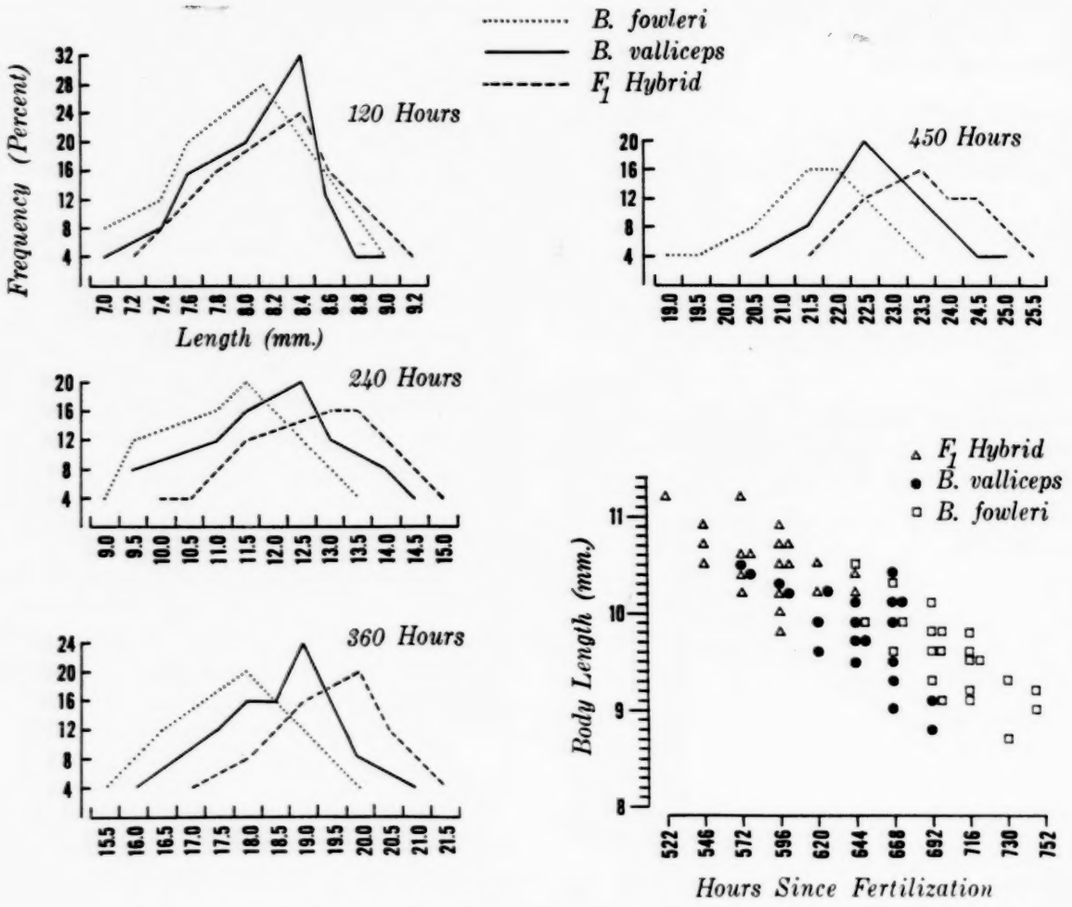


FIG. 1. Comparative rates of development under uniform conditions.

viable hybrids are sterile males from the cross, *fowleri* ♀ × *valliceps* ♂. The F_1 hybrid toads possess cranial crests characteristic of the *valliceps* parent, a modified form of dorsal spotting inherited from the *fowleri* parent, and a unique reticulated pattern of pigment on the ventral surface, which is not found in either parental species. The artificial production of hybrids permitted an unequivocal identification of F_1 hybrids in nature.

The F_1 hybrid tadpoles can also be distinguished on a morphological basis, thus furnishing an excellent means of testing the relative growth and survival of hybrid and non-hybrid larvae. The F_1 hybrid larvae are characterized by a *fowleri*-like pigmentary pattern on the tail musculature and a *valliceps*-like distribution of melanophores on the tail fins.

The precise knowledge of the larval and adult features of the constituent individuals of the local hybrid population provided the foundation for an elaborate series of studies, reported here, on the fate of the hybrids and the net effect of hybridization on the population structure of each parental species.

HYBRID VIGOR

Comparative rates of development under uniform laboratory conditions. Heterosis is suggested by the observation that the hybrid larvae are larger and transform earlier than larvae of either parental species. The three kinds of larvae (*valliceps*, *fowleri*, and F_1 hybrid) were reared under uniform, controlled conditions so that differences in growth rates and size could be ascribed to differences in genetic constitutions. The technique described in detail by Limbaugh and Volpe (1957) was employed.

Briefly, a total of 75 larvae or 25 from each of three crosses conducted simultaneously (*valliceps* ♀ × *valliceps* ♂, *fowleri* ♀ × *fowleri* ♂, and *fowleri* ♀ × *valliceps* ♂) were isolated in separate covered enamel pans containing pond water. The 75 pans were kept in "Precision Scientific" constant temperature units set at 25° C. The pond water in the individual pans was changed daily, the water having been brought to 25° C. prior to the change. Each larva was provided with more food (standard diet of boiled spinach) than could be utilized in a day. Size determinations were made at specific intervals by anesthetizing each larva with "M.S.222" (Sandoz Chemical Company, New York City) and measuring the distance between the snout and the tip of the tail to the nearest 0.01 mm with a Filar micrometer eyepiece inserted into a stereoscopic microscope.

The lengths of the 75 larvae (25 *valliceps*, 25 *fowleri*, and 25 F_1 hybrids) were measured and recorded at ages of 120, 240, 360, and 450 hours after fertilization. The results are presented in figure 1 in the form of frequency polygons. The abscissa represents the lengths of the larvae in millimeters; the ordinate, the percentages of larvae in the various size classes.

At 120 hours, the larval mouthparts were differentiated, feeding activity had commenced, and the hind limb rudiment was a small, undifferentiated bud (stage 26, defined in Limbaugh and Volpe, 1957). During this early larval period, the tadpoles were not distinguishable from each other on the basis of size. The dimensions of the hybrid larvae and of the parental larvae were approximately alike (upper left hand graph in fig. 1). The average length of *fowleri* larvae was 8.07 mm ± 0.10 (mean ± standard error); *valliceps*, 8.16 mm ± 0.09; and F_1 hybrid, 8.19 mm ± 0.11.

At the age of 240 hours, the growth of the hybrid larvae was clearly more advanced than that of each parental type. The hybrid larvae averaged 13.03 mm ± 0.24 (mean ± standard error); *valliceps*, 12.29 mm ± 0.25; and *fowleri*, 11.54 mm ± 0.23. The differences in lengths between the three groups are regarded as significant. The difference in mean values between the hybrid and *valliceps* larvae is twice as large as the standard error of the difference (0.74/0.35 = 2.1); the difference in the means between *valliceps* and *fowleri* is also twice as large as its standard error (0.75/0.34 = 2.2). Differences of this kind might arise by chance in 3.57 and 2.78 per cent, respectively, of similar comparisons. The accelerated growth of the hybrid larvae was also evident at subsequent ages (fig. 1). At 360 and 450 hours, statistically reliable differences exist between the three groups, comparable in magnitude to the differences at 240 hours. The smallest larvae were invariably those of *fowleri*.

Repeated observations were made as the larvae approached metamorphosis, in order to determine accurately the times at which the larvae completed transformation into juvenile toads. As shown in figure 1 (lower right hand graph), the hybrids transformed into young toads earliest, and, as expected, were largest in body size (snout-to-vent length). The rapid hybrid growth is exemplified by the following calculation based on the data graphically represented. At the time (596 hours) that 81 per cent of the hybrid larvae had completed transformation, only 19 per cent of the *valliceps* larvae and none of the *fowleri* larvae had undergone metamorphosis. Considering body lengths at metamorphosis, the juvenile hybrids (mean ± standard error: 10.51 mm ± 0.08) differ significantly in size from those of *valliceps* (9.82 mm ± 0.10) and of *fowleri* (9.57 mm ± 0.09). The latter two are not significantly different from each other (P = 0.06).

Comparative rates of development under competitive conditions in the laboratory and in the field. Five attempts were made to compare the growth rates

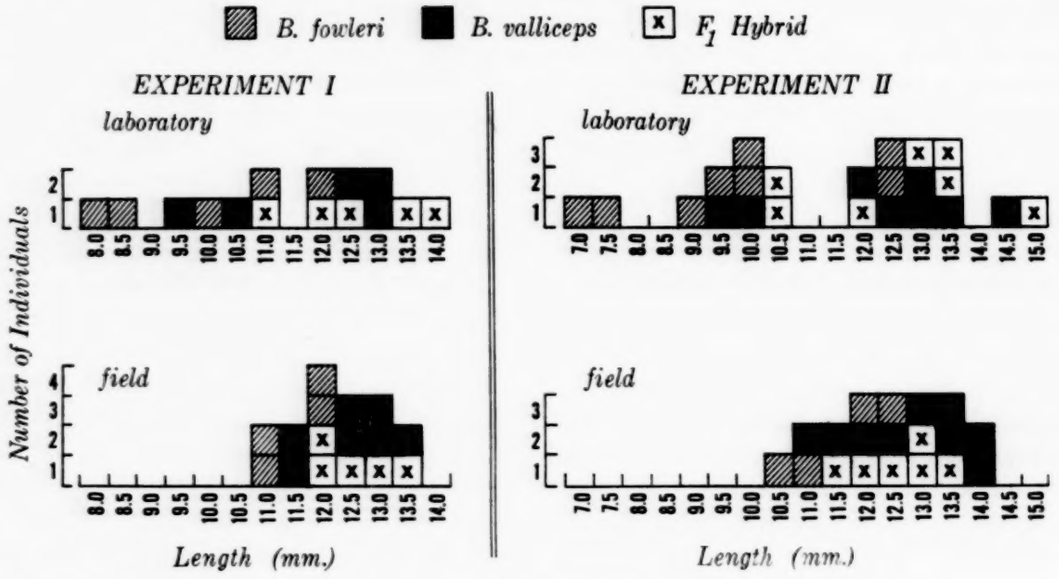


FIG. 2. Comparative rates of development under competitive conditions in the laboratory and in the field (two experiments). In each experiment, larvae were measured and identified 10 days after fertilization.

of the three kinds of larvae (*valliceps, fowleri,* and F_1 hybrid) in crowded culture conditions in the laboratory and in a confined area in the natural habitat at Aububon Park. Embryos were introduced into a small ditch in a relatively undisturbed area in the park. Only two of the five trials were successful. On one occasion, the temporary water in the ditch evaporated; on two other occasions, the ground surface adjacent to the ditch became flooded by heavy rainfall, providing (presumably) avenues of dispersal for the tadpoles.

In the first of the successful trials, the lengths of 45 larvae (15 *valliceps,* 15 *fowleri,* 15 F_1 hybrid) were measured 120 hours after fertilization. The average dimensions of the three kinds of larvae at 120 hours did not differ significantly from each other, verifying the results of the experiment described in the preceding section. These 45 larvae were then distributed. Fifteen larvae (5 *valliceps,* 5 *fowleri,* 5 F_1 hybrids) were reared together at room temperature in pond water in 12 by 8 by 2-inch white enamel pans. The remaining thirty larvae, ten of each kind, were transferred to the ditch at Audubon Park. Five days later, the larvae in the enamel pans as well as those recovered from nature were measured, preserved, and identified. As mentioned earlier, the larvae are readily identifiable on the basis of pigmentary differences.

The second successful experiment was identical to the first, except that the competitive conditions were made more stringent—24 larvae (8 of each kind) were reared in the laboratory in a single enamel pan and 48 (16 of each kind) were placed in the natural habitat. The results of both experiments are shown in histogram form in figure 2.

The laboratory-reared larvae varied considerably in size, particularly under the more adverse conditions of the second experiment. Undoubtedly, many tadpoles failed to obtain sufficient food in competition with others. The rapid growth rate of the F_1 hybrid larvae witnessed in isolated cultures is not particularly marked under competitive conditions. The larger-sized larvae included both *valliceps* and F_1 hybrids. The *fowleri* larvae exhibited delayed growth.

The most striking aspect of the larvae recovered from nature was the narrow range of variation of sizes of the larvae.

This finding is interpretable in two different ways. It may be that the growth rates are simply more uniform in nature than under laboratory conditions, notwithstanding the more constant environment in the laboratory. Alternatively, the growth rates may be comparable under both sets of conditions, in which case the apparent reduction in variability in nature may reflect differential selection against the smaller-sized larvae (and possibly the larger-sized larvae!). In other words, the extreme variants, departing far from the "average" growth rate, were eliminated. This may, in part, explain the predominance of *valliceps* larvae. The growth rate of *valliceps* is intermediate between those of *fowleri* and the F_1 hybrid, and may be optimum for the prevailing environmental conditions. Of the 16 recovered in the first experiment, 7 were *valliceps*, 5 were F_1 hybrids, and 4 were *fowleri*. Of the 19 recovered in the second experiment, 9 were *valliceps*, 6 were F_1 hybrids, and 4 were *fowleri*.

Whatever may be the factors responsible for the decreased variability in nature, it is apparent that the F_1 hybrid larvae can maintain themselves in considerable numbers in competition with *valliceps* larvae and that the *fowleri* larvae are adversely affected by the presence of both *valliceps* and F_1 hybrid larvae.

The Natural Hybrid Population

Frequency of mis-mating. The breeding site at Audubon Park was surveyed systematically for three successive years, 1956–1958. There is no sharply delineated breeding season, but rather several rain-induced breeding periods. Commencing in late March, extensive choruses may be heard virtually any evening following approximately an inch of rainfall. Mixed congregations of *valliceps* and *fowleri* occur from late March to early June. *B. fowleri* is most active in late March and April, less conspicuous in May and June, and absent from breeding choruses in July and August. *B. valliceps* is not only as active as *fowleri* during the early spring, but continues its breeding activity with a high degree of intensity into July and August. F_1 hybrids emerge early in the season; no hybrid has been found during the summer months. Hence, breeding congregations consist either of individuals of the two species and their hybrids or only of *valliceps*.

On 20 evenings following heavy rainfall during the three-year study period, counts were made of the number and types of toads participating in the breeding congresses, the number of normal matings, and the number of mis-matings. Observations were made between 8:00 P.M. and 10:00 P.M. of each evening. The data are recorded in table 1. *B. valliceps* invariably comprised the bulk of the breeding population, typically outnumbering *fowleri* by 3:1. In most instances, F_1 hybrids were found. Very rarely was a female *in the pool* encountered without a mate.

The figures presented in the following discussion of the data pertain to the "grand total" (see table 1) for the three-year period. In terms of actual numbers, more *valliceps* females were mis-clasped by *fowleri* males than reciprocally. However, in terms of percentages, only 6.5 per cent of the *valliceps* females were mis-mated by *fowleri* males, whereas 15.2 per cent of the *fowleri* females were mis-clasped by *valliceps* males. The F_1 hybrids displayed a propensity for *fowleri* females. Seven, or 15.2 per cent, of the *fowleri* females were embraced by F_1 hybrids; only one, or 0.5 per cent, of the *valliceps* females was mated by an F_1 hybrid. In essence, only 69.6 per cent of the *fowleri* females were mated by members of their own species, in sharp contrast to the 92.9 per cent of *valliceps* females that were normally mated. The 27 mis-clasped pairs (interspecific and F_1 crosses) represent 11.7 per cent of the total number of clasping pairs recorded.

The F_1 hybrids constitute 1.7 per

cent of the total population. The vigor of the F_1 hybrid is reflected in the high number that succeeded in finding a mate. Seventeen per cent of the F_1 hybrids were found in amplexus with females of both species, although, as indicated above, much more often with *fowleri* females. The figure gains significance when one considers that 8.5 per cent of the *valliceps* males found normal mates and only 6.2 per cent of the *fowleri* males located females of their own kind.

Recoveries of marked individuals. Certain individuals in the natural population were marked for future recognition. Males and females engaged in amplexus and F_1 hybrids were marked on 13 observation nights in 1956 and 1957 by the toe clipping method described by Martof (1953). The mated pairs and F_1 hybrids were captured by hand, particular digits were cut with cuticle scissors, and the individuals involved were released at the capture site. Dur-

TABLE 1. *Number and mating activity of toads in the hybrid population at Audubon Park, New Orleans. The counts represent the number and sex of* B. valliceps, B. fowleri, *and F_1 hybrids observed, and the number of normal matings and mis-matings recorded on 20 evenings during the three-year perod, 1956–1958. The two numbers in parentheses below each collection date are the total inches of rainfall and the air temperature (°F) at the breeding site at 8:00 p.m., respectively*

Date	Adult *fowleri*		Adult *valliceps*		Adult F_1 hybrids	Matings of *fowleri* ♀ ♀			Matings of *valliceps* ♀ ♀		
	♂♂	♀♀	♂♂	♀♀	♂♂	× *fow.* ♂	× *val.* ♂	× hyb. ♂	× *val.* ♂	× *fow.* ♂	× hyb. ♂
1956											
April 9 (1.79)(68°)	21	2	58	4	4	1	0	1	3	1	0
April 24 (1.30)(63°)	46	5	152	14	3	3	1	1	12	2	0
May 2 (1.19)(70°)	39	3	142	18	2	2	1	0	17	1	0
May 5 (0.94)(72°)	22	2	84	5	1	2	0	0	5	0	0
May 23 (0.87)(79°)	10	1	67	6	0	1	0	0	6	0	0
May 26 (2.43)(76°)	8	1	108	8	1	0	1	0	8	0	0
Totals	146	14	611	55	11	9	3	2	51	4	0
1957											
March 21 (1.46)(66°)	28	4	65	6	2	3	1	0	6	0	0
March 31 (2.86)(64°)	42	4	176	16	5	3	0	1	14	2	0
April 4 (1.98)(63°)	25	3	194	12	5	1	1	1	11	1	0
April 11 (1.03)(61°)	39	2	87	10	2	2	0	0	9	0	1
April 17 (1.76)(68°)	32	2	112	10	2	1	0	1	9	1	0
April 30 (1.00)(70°)	13	1	60	8	1	1	0	0	8	0	0
May 2 (0.72)(70°)	12	1	54	5	0	1	0	0	5	0	0
Totals	191	17	748	67	17	12	2	3	62	4	1

TABLE 1—*Continued*

Date	Adult *fowleri*		Adult *valliceps*		Adult F_1 hybrids	Matings of *fowleri* ♀ ♀			Matings of *valliceps* ♀ ♀		
	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂	× *fow.* ♂	× *val.* ♂	× hyb. ♂	× *val.* ♂	× *fow.* ♂	× hyb. ♂
1958											
April 9 (1.14)(61°)	41	3	96	7	4	2	0	1	6	0	0
April 14 (1.20)(62°)	35	4	102	7	3	3	1	0	6	1	0
May 10 (2.10)(67°)	43	6	139	21	5	4	1	1	19	2	0
May 11 (1.00)(75°)	29	1	67	5	1	1	0	0	5	0	0
May 18 (1.28)(73°)	20	1	108	10	5	1	0	0	10	1	0
June 6 (0.83)(86°)	11	0	92	7	1	0	0	0	7	0	0
June 15 (0.99)(84°)	3	0	67	6	0	0	0	0	6	0	0
Totals	182	15	671	63	19	11	2	2	59	4	0
Grand total	519	46	2,030	185	47	32	7	7	172	12	1

ing the handling of the mated pairs, the males clung tenaciously to their partners.

The purpose of this study was to ascertain, firstly, whether F_1 hybrids survived from one year to the next, and, secondly, whether females mis-mated in one year would be, if recaptured in subsequent years, mis-mated again. The data are summarized in table 2.

More than half of the F_1 hybrids captured and marked in any one year were recovered the subsequent year, and one-third of the hybrids marked in one year were recaptured two years following. Two of the six hybrids that had clasped females in 1956 and 1957 were subsequently recovered, but both were unmated when recaptured. One unmated hybrid marked in 1957 was found in amplexus with a *fowleri* female in 1958.

Only four per cent of the *valliceps* females that had been mated in 1956 were recaptured in amplexus in 1957. Of the mated females marked in 1957, a comparably small number (five per cent) was recaptured in 1958. Moreover, very few *valliceps* males that had successfully bred in one season were found in amplexus again in the subsequent season. None of the recovered *valliceps* individuals was mis-mated.

The reappearance of marked *fowleri* females contrasted sharply with the rare recurrences of marked *valliceps* females. Evidently, there is considerably less turnover of individuals in the *fowleri* population than in the *valliceps* population. Of the 11 *fowleri* females captured and marked in 1956, six had been mated normally and five had been mis-mated. Four of these 11 females were recovered in 1957, and all four were clasped by males of their kind. Interestingly, the four re-mated *fowleri* females were of the group that had *not* been mis-mated the previous season (1956). The probability of recovering only females that had been previously mated normally is $(6/11)^4$ or 1 in 11. The odds against a chance occurrence are increased by the subsequent finding that two of the four normally-mated females were recaptured

TABLE 2. *Recoveries of marked toads in the hybrid population at Audubon Park, New Orleans. The number of F_1 hybrids and mated individuals of* B. valliceps *and* B. fowleri *originally marked in 1956 and 1957 are given in the first column; the number of each kind recaptured in subsequent years is indicated in the second and third columns*

	Recoveries	
	1957	1958
* F_1 hybrids		
1956: 9 ♂♂	5	3
1957: 11 ♂♂	—	6
* *B. valliceps*		
1956: 51 ♀♀	2	0
50 ♂♂	3	1
1957: 62 ♀♀	—	3
59 ♂♂	—	2
* *B. fowleri*		
1956: 11 ♀♀	4	2
10 ♂♂	2	0
1957: 12 ♀♀	—	3
13 ♂♂	—	2

* The fewer numbers of breeding individuals marked than observed (see table 1) is due to the removal from the natural population of several F_1 hybrids and mated pairs of each species for experimental purposes (laboratory crosses and analyses of gonads).

in 1958, and, once again, in amplexus with *fowleri* males.

The situation was quite similar for females marked in 1957 and recovered in 1958. Of the seven *fowleri* females with normal mates that were marked in 1957, three were found in 1958 with *fowleri* male partners. None of the five mis-mated *fowleri* females marked in 1957 was recaptured in 1958. In this circumstance, the probability of recovering only normally-mated females is $(7/12)^3$ or 1 chance out of 5.

The lack of recovery of mis-mated *fowleri* females in two successive years is striking, and, compounding the individual probabilities ($1/11 \times 1/5 = 1/55$), is not likely to be due to chance alone. The fate of the mis-mated females is speculative. What has been demonstrated is that those females that accept a foreign male in one year are not found in amplexus in subsequent years. These females may still be in the population, but in terms of contributing to the gene pool of subsequent generations, they have been effectively eliminated.

Counts of egg strands and their significance. The numerical picture of the population presented above is at best a crude representation of the structure of the hybrid population. The census of mated pairs taken any given evening during a two-hour period is obviously not a true valuation of the number of matings that actually occurred during an evening of breeding. This was revealed vividly by counts of egg strands noted in the breeding habitat the day following the evening activity. The eggs of a single female, each approximately 1 mm in diameter, are laid in two strands, like two strings of beads. Unless there has been considerable breeding activity, the double egg strands of different matings can be distinguished from one another. If undisturbed, a female will deposit all her eggs in a narrowly circumscribed area. The least amount of disturbance of the habitat is obviously to be found in the first evening of breeding activity of the season. Consequently, the collecting phase of this particular study was undertaken the afternoon following the first period of breeding in each of three seasons, namely, April 10, 1956, March 22, 1957, and April 10, 1958. The uniqueness of the laboratory phase of this study is that an examination of the embryos derived from a given double egg strand leads to a positive identification of the parents.

On each of the three aforementioned dates, approximately 25 eggs were removed from each double egg strand found in the breeding area. Each sample of eggs was placed in pond water in a white enamel pan. Thirteen samples were obtained on April 10, 1956; fifteen on March 22, 1957; and thirteen on April 10, 1958. The outcome of all possible combinations of crosses is shown in

figure 3. If the eggs are unfertilized, then a *valliceps* or *fowleri* female has been mis-clasped by an F_1 hybrid; if the eggs develop into abnormal gastrulae with extruding yolk plugs, then a *valliceps* ♀ has been mis-mated by a *fowleri* ♂; and, if the eggs develop into functional larvae, then either of three crosses has occurred (*valliceps* ♀ × *valliceps* ♂, *fowleri* ♀ × *fowleri* ♂, or *fowleri* ♀ × *valliceps* ♀). The larvae resulting from the latter three crosses are readily distinguishable, as illustrated in figure 3 and described in detail in Volpe, 1956.

The results of the study are also tabulated in figure 3. Whereas only three mated pairs of *valliceps* were recorded on the evening of April 9, 1956, the examination of larvae that developed from eggs sampled the following afternoon revealed that 8 *valliceps* matings had actually occurred. Likewise, as judged by the egg samples, one additional *fowleri* mating had taken place

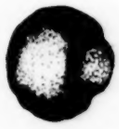
Cross	Outcome	Number of Matings April 9 1956	March 21 1957	April 9 1958
valliceps ♀ x *valliceps* ♂		8	10	10
fowleri ♀ x *valliceps* ♂		0	1	0
fowleri ♀ x *fowleri* ♂		2	4	2
valliceps ♀ x *fowleri* ♂		2	0	0
valliceps ♀ or *fowleri* ♀ x F_1 *hybrid* ♂		1	0	1

FIG. 3. The outcome of all possible combinations of crosses involving *B. valliceps*, *B. fowleri*, and F_1 hybrids is illustrated. The numerals refer to the number of matings that occurred in three evenings of breeding activity as revealed by the rearing and identification of embryos that developed from egg strings taken from the natural hybrid population (Audubon Park, New Orleans) the afternoon following the evening of breeding activity.

and one additional mis-mating (*valliceps* ♀ × *fowleri* ♂) had occurred during the evening of April 9, 1956. The one F_1 hybrid mating (*fowleri* ♀ × F_1 hybrid) recorded during this evening was revealed by a single group of unfertilized eggs among the 15 samples.

The examination of embryos derived from eggs sampled on the afternoons of March 22, 1957 and April 10, 1958, disclosed essentially the same situation. Approximately twice as many *valliceps* matings had occurred than were recorded during the previous evenings of breeding activity and an additional *fowleri* mating had taken place, but no other mis-matings had occurred. Those mis-matings observed during the respective two evenings were detected in the egg samples.

The additional matings uncovered in this study may be expressed in terms of average increments. Roughly, 2.0 times as many *valliceps* matings probably had occurred each evening than were recorded by the census taken during a limited period of observation; 1.5 (a conservative figure) times as many *fowleri* matings probably had taken place; and 1.1 times as many mis-matings (interspecific and F_1 crosses) probably had occurred. Based on these average increments, a better estimate of the total number of mated pairs during the three-year period would be 344 *valliceps* matings, 48 *fowleri* matings, and 30 mis-matings (compare with figures under "grand total" in table 1). Thus, instead of the mis-clasped pairs representing 11.7 per cent of the total number of clasping pairs (as calculated from total counts, table 1), the revised figure would be 7.1 per cent. This still represents a high frequency of mis-matings in a natural population!

Discussion

The sterile condition of the F_1 hybrid male, the only viable offspring resulting from interspecific crosses between *B. fowleri* and *B. valliceps*, is not a sign of a general weakness. On the contrary, the present data reveal that the hybrid is equal, if not superior, in somatic vigor to either parental form. Heterosis is indicated by the rapid growth rate and large size of the F_1 hybrid larvae. In isolated cultures, the hybrid larvae develop faster and metamorphose earlier and at a larger size than those of either parental species. Under competitive conditions in mixed cultures, both in the laboratory and in nature, the accelerated growth of the hybrid larvae is not as pronounced, but the hybrid larvae do compete successfully with both parental larvae.

In the natural hybrid population studied for a three-year period, the majority of the adult hybrids captured and marked in any one season have been recovered the subsequent season, and several have been recaptured two seasons following. Unmarked hybrids have been collected each season, either constituting new arrivals or individuals that had escaped detection in the previous season. It seems unlikely that the hybrids survive by sheer chance. Their reappearance year after year probably reflects their sturdy nature.[2] Although sterile, the hybrids are very active in breeding congregations and do mate with females of either parental species, although more often with *fowleri* females.

Hybridization between the parental types results in a complete wastage of reproductive energy, and it is now evident that the sterile, but vigorous, F_1 hybrids are causing further losses of gametes from the gene pools of the parental species. To what extent has this ill-fated sequence of hybrid events affected the population characteristics of each species? The contention is held that only the *fowleri* segment of the hybrid population is being significantly affected. The lines of reasoning are developed below.

[2] In Texas populations, as demonstrated by Thornton (1955), *B. valliceps* hybridizes with *B. woodhousei*, a species closely related to *B. fowleri*. The *woodhousei-valliceps* hybrids appear also to be sturdy. Data by W. F. Blair (1956) indicate that the survival in nature of the *woodhousei-valliceps* hybrids is at least equal to that of the parental *valliceps*.

It should be first appreciated that *B. valliceps* is better adapted to the Gulf Coast environs than is *B. fowleri*. *B. valliceps,* a neotropical species, is the dominant toad in the "deltaic plain unit" and the "Mississippi River alluvial plain unit" of Louisiana, physiographic divisions characterized by marsh lands as described by Holland (1944). This toad breeds in temporary bodies of water, such as roadside puddles, irrigation ditches, and rain-flooded meadows. The larvae possess a rate of development that is correlated with the limited period of time available for growth in temporary pools. Interpreting the results of the present investigation, the growth rate approaches the optimum for the prevailing environmental conditions.

B. fowleri is widely distributed in the eastern half of the United States. The Louisiana populations of this species lie at the periphery of the distributional range. In the deltaic regions of Louisiana, *B. fowleri* is far less abundant than the Gulf Coast toad, which reflects in part the unsuitability of breeding habitats. Members of this species ordinarily exhibit a preference for permanent breeding sites, such as stream borders and pond margins. The dearth of natural ponds in the Gulf Coast area has prohibited successful colonization of the area by this species. The development of certain adaptations to the local conditions has apparently not occurred, as witnessed by the slow rate of larval development. As suggested by the competition studies, there is selection against the slower-growing, smaller-sized *fowleri* larvae in nature.

B. valliceps invariably predominates in mixed breeding choruses, as in the hybrid population extensively studied at Audubon Park, New Orleans. The *valliceps* segment of the total population of toads is extremely large. There is, as disclosed by marking experiments, a rapid population turnover in a one-year period. Individuals that have mated in one season have rarely been recovered in the subsequent season. Females of this species have no difficulty in finding mates of their kind. Mis-matings by *fowleri* males or F_1 hybrids do occur, but it is doubtful if the consequent loss of gametes has impaired seriously the overall fitness of the *valliceps* assemblage. The gametic loss through hybridization is essentially absorbed by the reproductive potential of the large number of individuals. Moreover, mixed breeding choruses are present only from April to June. In July and August, *valliceps* continues its breeding activity with a high degree of intensity in the absence of *fowleri* and F_1 hybrid individuals. The *valliceps* gene pool, if drained earlier to any appreciable amount, is certainly replenished later in the season.

On the other hand, *fowleri* choruses in the Audubon population are always contaminated by *valliceps* individuals and F_1 hybrids. The *fowleri* segment of the population is maintained by a small number of individuals. Since it appears that only a few *fowleri* females perpetuate the stock each year, it is obviously important to the survival of the species that the females find their proper mates. However, *valliceps* males outnumber *fowleri* males by at least three to one, and quite often, the *fowleri* females are mis-mated by either *valliceps* males or F_1 hybrids. If the high frequency of mis-matings is continued unchecked, then an extreme diminution of the *fowleri* population should be witnessed. However, it appears that *fowleri* individuals can maintain themselves, even if only in small numbers, at least during the three-year period studied.

A re-check of the population ten or twenty years hence will obviously provide greater insight into the evolutionary dynamics of the population. However, the present three-year study permits certain conjectures.

It may be that the population size of *fowleri* remains fairly constant from year to year. Several factors may operate to maintain a limited population size. As the population density rises, individuals compete more intensely for means of sub-

sistence and mates. The activity of predators and parasites is also intensified. Finally, mis-matings occur, which, through inviability and sterility of the offspring, sharply curtail the tendency toward increase in numbers. In essence, only a limited number of females can successfully reproduce annually. Viewed in this light, interspecific matings constitute a very effective control of population size.

Examined from another point of view, extensive hybridization may be leading to a reconstruction of the gene pool of *fowleri,* resulting in a species genotype of a higher adaptive value. It is noteworthy that the *fowleri* females that have been recovered in subsequent years were those that had *not* been mis-mated the previous year. The *fowleri* females that had been mis-clasped in one season were *not* found in amplexus the following season. It is unlikely that the lack of recovery of mis-mated *fowleri* female is due to chance alone. It is quite plausible that *valliceps* and the sterile F_1 hybrids are effectively removing through mis-matings the ill-adapted *fowleri* females and leading to the maintenance and multiplication of *fowleri* individuals better fitted for the given conditions of existence and reproduction. It is generally accepted that "whether a mating will be homospecific or heterospecific in a mixed population depends on the discrimination of the female" (W. F. Blair, 1958). Those females that resist most successfully the attentions of males of the opposite species may be the ones carrying the most favorable genotypes.

If there is and continues to be differential propagation of *fowleri* genotypes, then selection will serve to strengthen isolation between sympatric species whose complexes are exposed to the risk of disintegration, as contended by Dobzhansky (1940) and espoused recently by W. F. Blair (1958). It is stressed that isolation will be reinforced only if there are significant genetic differences between those individuals that find normal mates and those that are mis-mated. The distinguishing heritable traits, if any, of normally-mated and mis-mated *fowleri* females are not known.

Summary

Hybridization between *B. valliceps* and *B. fowleri* results in a wastage of reproductive energy and food resources, since the F_1 hybrids are either inviable or sterile males. The sterile F_1 hybrids are somatically vigorous and maintain themselves in relatively high numbers in nature. Further losses of gametes occur when the sterile F_1 hybrids mate with the parental females, although more often with *fowleri* females, and cause them to extrude their eggs. In the local hybrid population, intensively analyzed, the data indicate that the gene pool of the abundant, highly adapted *B. valliceps* assemblage is not being seriously impaired by the high frequency of hybridization. However, it appears likely that the less numerous, less adapted *B. fowleri* segment of the population is being affected by the extensive hybridization. The mis-matings may serve as a potent factor operating to keep the *fowleri* population at a constant, limited density. It is also conceivable that ill-adapted *fowleri* individuals are being effectively eliminated through mis-matings and supplanted by individuals better fit for the particular local conditions of existence and reproduction. The critical information on the distinguishing heritable features, if any, of normally-mated and mis-mated *fowleri* females is lacking.

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THE CHORDA TYMPANI AND MIDDLE EAR AS GUIDES TO ORIGIN AND DIVERGENCE OF REPTILES[1]

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INTRODUCTION

The tetrapod middle ear originated by modification of parts of the jaw mechanism of fishes, and its subsequent history is closely associated with the history of the jaw apparatus. Its early evolution is a complex of changes induced by two different influences. On one hand there were modifications of jaw structure that forced changes upon the middle ear, and on the other there was a tendency for modification of middle ear structure toward refinement of hearing, or for maintenance of hearing in the face of alterations induced by structural changes of the jaw. All of these changes were adaptive in nature.

The chorda tympani is a small branch of the facial nerve that passes through the tympanic cavity on its way to the lingual side of the lower jaw. Its relationships to the various parts of the middle ear are remarkably consistent among living tetrapods. Position of the nerve has nothing to do with function of either the middle ear or the jaw mechanism and so is unlikely to have been affected by functional adjustments of the middle ear. Therefore the consistency of its relationships is generally thought to be a conservative feature, one that may be of value in determining patterns of phylogeny, and its position has been extensively studied in living forms. Unfortunately the nerve has left little trace of its position in fossil forms, which have therefore been largely ignored in such studies.

Also because of its poor record chorda tympani has seldom been considered in studies of relationships among fossil tetrapods, even though the controversy over the origin and radiation of reptiles currently centers on the nature of the middle ear.

A reinterpretation of the pelycosaur stapes (Hotton, 1959) has led to recognition of features that mark the route of the chorda tympani in very primitive reptiles. The present paper is an attempt to correlate this information with the abundant material on the chorda tympani in modern forms, in order to demonstrate that the position of the nerve in the pelycosaurs represents a logical intermediate between the condition of fish and amphibians on one hand and all amniotes on the other, and to show that changes of the middle ear from fish to modern amniotes can reasonably be documented by known fossil forms.

GENERAL MORPHOLOGY

Otic region. The essential parts of the middle ear consist of a more or less superficial membrane, the tympanum; a small bone, the stapes, which connects the tympanum with the fenestra ovalis in the wall of the otic capsule; and an air-filled chamber, the tympanic cavity. The tympanic cavity communicates with the pharynx proximally and is closed by the tympanum distally; it surrounds the stapes and is delimited in part by various bones of the occiput and cheek region. This general pattern is expressed in all living tetrapods except urodele and caecilian amphibians, snakes, *Sphenodon*, and some lizards. It is modified in mammals by the interposition of two small bones of lower jaw origin, the malleus and incus, between tympanum and stapes.

In discussions of anurans, birds, and

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living reptiles the stapes is usually described as consisting of two parts, a proximal bony oto-stapes (columella auris) and a distal cartilagenous extra-stapes (extracolumella); the later is in contact with the tympanum and bears one or more additional processes that extend variously to the bones surrounding the ear cavity. The processes are thought to be homologous among the tetrapods, and Eaton (1939) has demonstrated in a general way the homology of the processes of the tetrapod ossicle with those of the crossopterygian hyomandibular. Most of the processes known in the modern forms only as cartilagenous or ligamentous branches of the extra-stapes are preserved as bone in primitive fossil tetrapods. In order that comparison between the ossicles of living and fossil reptiles may be facilitated, the term stapes as used in this paper includes both oto-stapes and extra-stapes. Dorsal process designates the connection to the opisthotic, tympanic process the connection to the tympanum, and internal process the connection to the quadrate.

Bony parts of the middle ear of frogs are similar in most respects to those of fossil labyrinthodont amphibians. In the latter forms the ear is delimited by the otic notch, a posterior concavity that lies high on the back margin of the skull and involves the squamosal, supratemporal and tabular bones. The moderately massive stapes is directed toward the notch, which presumably housed a tympanum as it does in the frog. Most primitive reptiles of late Pennsylvanian and early Permian age are characterized by loss of the otic notch. In these forms the stapes is directed toward the articular end of the quadrate bone and is usually quite massive. A few early fossil reptiles (*Diadectes* and its close relatives) have a large otic concavity which may be, but is not necessarily, a retention of the amphibian otic notch. The stapes is not well known in the diadectids, but appears to be proportionately smaller than that of any contemporary form without an otic notch. In all more advanced reptiles and in birds the tympanum (when present) is supported in a concavity behind the quadrate near the jaw joint. Only secondarily (in crocodiles and some dinosaurs) does it occupy a position high on the skull. The stapes is always reduced to a slender spicule in advanced forms.

Chorda tympani. The trunk of the facial nerve emerges from in front of the otic capsule to traverse the membranous lining of the tympanic cavity in anurans, reptiles, and birds. It passes backward above the stapes and then downward in the posterior wall of the cavity. Its course is similar in mammals, but it is enclosed in the bony wall of the middle ear. The internal mandibular branch, chorda tympani, arises from the main trunk of VII behind the stapes. In the frog its entire downward course lies in the back wall of the middle ear; it passes forward to the lower jaw below the tympanic cavity. In modern reptiles, on the other hand, chorda tympani passes forward above the distal end of the stapes to run down the anterior wall of the tympanic cavity in front of the ossicle; it reaches the lower jaw via the posterior surface of the quadrate bone. In mammals chorda tympani passes from the back wall to the front wall on the medial surface of the ear drum. Its precise relationship to the distal end of the stapes is undeterminable because the mammalian stapes does not extend to the ear drum, but the nerve does not pass below the tympanic cavity as in the frog.

Problems of homology. Understanding of relationships of chorda tympani in the various tetrapod groups is dependent in part upon knowledge of the development and homology of the parts of the middle ear, in particular the tympanic cavity itself. Prior to the work of Goodrich (1915), the tympanic cavity of all tetrapods was considered to be homologous with the spiracular cleft of fishes. Goodrich demonstrated the distinctiveness of the amniote ear cavity by showing that it arises embryonically as a postero-ventral diverticulum of the spiracular pouch in

such a way that chorda tympani comes to lie in front of the diverticulum. He regarded the post-tympanic position of chorda tympani in the frog as a survival of an ancestral condition (Goodrich, 1930) in which the tympanic cavity is still essentially homologous with the spiracular cleft. DeBeer (1937) differed with this interpretation, holding that the condition in the frog is an embryonic specialization related to the anterior placement of the first two visceral arches in the tadpole, and that the frog tympanic cavity is homologous with that of amniotes, not with the spiracular cleft. However, the anurans are a very restricted and specialized group, and the peculiar events of their metamorphosis render embryological evidence unreliable in interpreting homologies of the amphibian tympanic cavity.

For further information on the amphibian ear one must turn to primitive representatives of the fossil labyrinthodonts. These animals resemble anurans and are in contrast with reptiles in the high position of their tympanic cavity, and Parrington (1958) suggests that this feature indicates true homology between the amphibian ear cavity and the fish spiracular cleft, and that chorda tympani was post-tympanic in the labyrinthodonts as well as in anurans.

The labyrinthodonts are divided by Romer (1947) into two orders, the Temnospondyli and the Anthracosauria. The Temnospondyli quite certainly contain the ancestors of frogs and toads (Watson, 1940, but see also Romer, 1947), which tends to support Goodrich's idea of the primitive nature of chorda tympani position in the frog. The Anthracosauria contain animals which, when judged on the basis of non-otic characters, provide excellent morphological antecedents for the reptiles. But the anthracosaur middle ear cavity is typically labyrinthodont, homologous with the spiracular cleft.

The impressive contrast between the labyrinthodonts and all other tetrapods in the middle ear has led Parrington and others to the conclusion that amniotes could not have descended from a labyrinthodont ancestor. Parrington recalls Sushkin's observation (Sushkin, 1927) that the stapes of very primitive reptiles, in its massiveness and orientation, resembles the hyomandibular of a fish more than it does the labyrinthodont stapes. Elaborating on Westoll's scheme (Westoll, 1943) that the tympanum originated behind the squamosal, he advances the concept of a "prototetrapod" in which the tympanum lay in a neutral position halfway between the dorsal labyrinthodont position and the ventral reptilian position, about where the operculum lies in a rhipidistian fish. According to this hypothesis, the spiracular opening in the skull was utilized as an otic notch and only the upper part of the fish hyomandibular became a functional stapes in the labyrinthodont middle ear. The amniote ear cavity was a separate development in a more ventral position; it utilized the greater part of the hyomandibular as a stapes, but there was no involvement of the spiracular cleft as a functional otic notch.

In view of the community of non-otic characters between the two groups, to throw the anthracosaurs out of reptile ancestry solely because of dissimilarity of middle ear structure partakes of throwing out the baby with the bath. It should be more economical, on the basis of current evidence, to reconcile the otic differences between the two groups.

Vaughn (1955), following Goodrich (1930), has attempted to do this by suggesting that chorda tympani was transferred from a post-tympanic to a pretympanic position via a hypothetical primitively thick tympanum. This theory is based on the premise that the nerve was primitively in a closer relation to the tympanum than to the stapes, which is open to question. In the crossopterygian fish chorda tympani passes through a canal in the hyomandibular before passing down to the medial side of the lower jaw. At no point, apparently, does it come into

contact with the putative future site of the tympanum. In lizards, which presumably show the most primitive middle ear arrangement among living amniotes, chorda tympani passes just lateral to and in contact with the dorsal process of the stapes, but not in contact with the tympanum. When the dorsal process is poorly developed or lacking (some lizards, all turtles), chorda tympani crosses above the stapes more medially. Only in birds and crocodilians, in which the dorsal process is very laterally placed, being itself in contact with the margin of the tympanum, does chorda tympani come into contact with the tympanum.

In the following sections a new interpretation of the pelycosaur stapes demonstrates considerable similarity between the labyrinthodont and pelycosaur middle ears. Functional necessity is adduced for claiming a labyrinthodont origin of the pelycosaur type of otic structure.

Morphology and Evolution of Primitive Middle Ears

The tetrapod stapes was derived from the hyomandibular bone of rhipidistian fishes; fig. 1 illustrates the probable mode of transition and is based upon the work of Romer (1941). In the fish, the hyomandibular was oriented with its proximal articulations (with the braincase) anterior and its distal articulations (with the quadrate) posterior. The facial nerve passed backward in the jugular canal (in the wall of the braincase) to a point between the two heads of the hyomandibular, and entered a canal within the hyomandibular itself via the medial surface of the bone. It passed through the proximal half of the bone within the canal and emerged on the lateral surface just below the opercular process. Transformation of hyomandibular into stapes involved reduction of ossification and rotation from a longitudinal to a transverse orientation, the bone pivoting on its cranial articulations as its distal end swung forward with the quadrate. The surfaces that had been lateral and medial in the fish became anterior and posterior, respectively, in the tetrapod. Bone covering the jugular canal disappeared so that the contents of the canal ran on the surface of the skull between the heads of

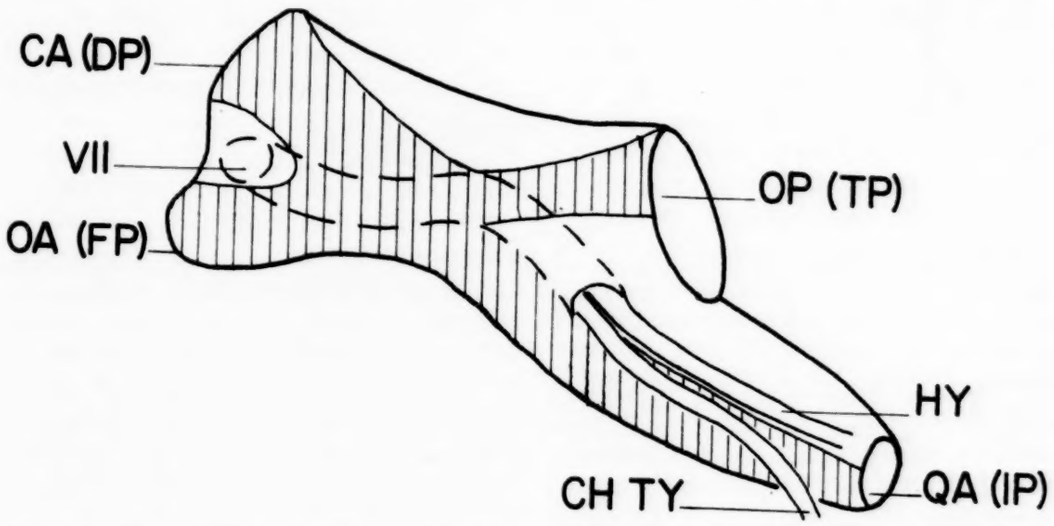

Fig. 1. Hyomandibular of *Ectosteorhachis* (*"Megalichthys"*) after Romer (1941). Lateral aspect, which is equivalent to anterior aspect of stapes. Lined portion indicates parts of element remaining after conversion to stapes. Reference letters (parenthetical terms refer to tetrapod homologues): CA (DP), cranial articulation (dorsal process); CH TY, chorda tympani; HY, hyoid branch of facial nerve; OA (FP), otic articulation (footplate); OP (TP), opercular process (tympanic process of amphibian only); QA (IP), quadrate articulation (internal process); VII, trunk of facial nerve.

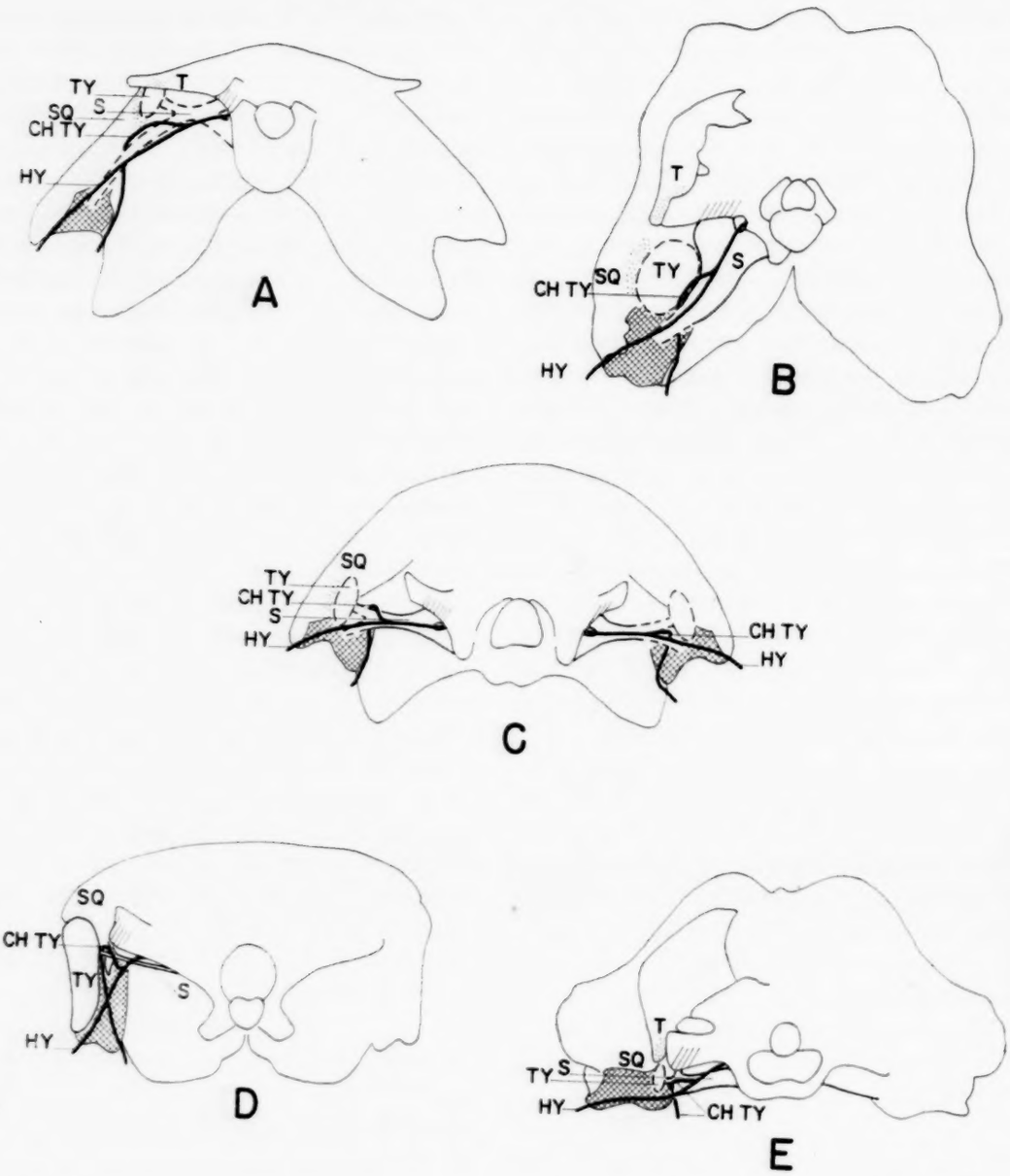

FIG. 2. Relationships of various parts of the middle ear at successive stages of tetrapod evolution. A, Amphibian stage. B, Primitive reptilian stage (immediately following otic notch closure). C, Advanced reptilian stage; left side shows neomorphic tympanic process arising lateral to chorda tympani, right side shows it arising medial to nerve. D, Definitive diapsid condition. E, Definitive therapsid condition. Reference letters: CH TY, chorda tympani; HY, hyoid branch of facial nerve; S, stapes; SQ, squamosal; T, tabular; TY, tympanum. Patterns: stippled, region of tympanum attachment (in B and D tabular stippling represents former tympanum attachment); lined, paroccipital process (region of dorsal process articulation); cross-hatched, quadrate bone (region of internal process articulation). A based on *Paleogyrinus* after Watson, 1926; B on *Dimetrodon* after Romer and Price, 1940; C on *Captorhinus;* D on *Crotaphytus;* E on *Dixeya* after Parrington, 1955.

the stapes. As the distal end of the ossicle moved forward, the back (formerly the medial) wall of the nerve canal disappeared, so that the bone lay entirely in front of the facial nerve to a point below the opercular process. Distally the ossicle remained behind (formerly medial to) the mandibular branch (chorda tympani),

and "pushed" it ahead as it pivoted. The upper half of the distal end of the bone was lost, permitting the hyoid branch of the facial to remain behind with the trunk of the nerve.

Labyrinthodont conditions. Figure 2, A is a restoration demonstrating the main features of the labyrinthodont middle ear. The skull is of anthracosaur pattern, specifically *Paleogyrinus;* it was selected because of its primitive nature, its great age, and its proximity to the stem of the reptilian stock. The anthracosaur stapes is not well known, and the shape of the ossicle shown in the diagram is based upon landmarks identifiable on the skull, and upon the better known temnospondyle stapes which it must have resembled. A ligamentous internal process must have descended from the functional axis of the stapes to the quadrate in the anthracosaur ancestors of reptiles as it is known to have done in some temnospondyls (Westoll, 1943; Watson, 1953). Evidence for the route of the labyrinthodont chorda tympani is based upon features of the rhipidistian hyomandibular and upon relationships in primitive pelycosaurs described below under "primitive reptilian conditions."

With loss of the bony operculum of the fish, the opercular process became the amphibian tympanic process; it lay above the point at which chorda tympani crossed the distal end of the ossicle, which had become the amphibian internal process. The primitive spiracular tympanic cavity surrounded only the upper part of the stapes at this stage; it did not enclose the internal process. Facial nerve and chorda tympani passed along the back wall and floor of the tympanic cavity, and mere loss of the internal process in temnospondylous ancestors would produce all details of the middle ear of frogs.

The otic notch of *Paleogyrinus* and other primitive anthracosaurs was wide open, probably as a holdover of the large space at the back of the skull that had been occupied by the operculum of the fish. If the tympanum originated from the tissue remaining after loss of the osseous operculum, one could expect it to be large and to occupy most of the otic notch in primitive forms. In fig. 2, A the stapes is reconstructed as having a high contact with the tympanum simply to emphasize the essentially labyrinthodont nature of the middle ear, but tympano-stapes contact could have occurred at an appreciably lower level in these animals, as suggested by Westoll (1943, fig. 3, C) and Parrington (1958, fig. 5). The greater distance from the otic capsule and low position of such a contact would require a stapes more reminiscent of the hyomandibular than that shown in my reconstruction.

Parrington's requirements of a low tympano-stapes contact and a large stapes for reptile ancestors then could be met by the anthracosaur middle ear, and one need not seek them among amphibians that lack an otic notch. Acceptance of these conditions makes no difference in interpreting the homology of the pre-reptile tympanic cavity. It would still lie medial to the tympanum and around the stapes, in the same morphological relationships found in other labyrinthodonts. As long as contact between stapes and membrane took place by means of the amphibian tympanic process (which was a direct derivative of the opercular process), chorda tympani must have run below the contact and therefore must have been post-tympanic in position. No matter how low the ancestral reptile tympanic cavity lay, it must still have been homologous with the spiracular cleft.

Future discoveries may reveal primitive anthracosaurs with massive stapes, but the fact that none have been found to date suggests that the anthracosaur stapes must have been a smaller, more easily-lost ossicle like that of other labyrinthodonts.

Primitive reptilian conditions (fig. 2, B). The stapes of primitive pelycosaurs and captorhinomorphs is massive and slants obliquely from the otic capsule to

FIG. 3. Left stapes of *Ophiacodon* in posterior aspect. Ossicle oriented naturally. Arrow points to notch for passage of chorda tympani across the stapes.

the articular region of the quadrate. It was probably attached to the quadrate. None of these stapes has a tympanic process; instead, the dorso-lateral margin of some of them (*Ophiacodon, Clepsydrops, Dimetrodon*) is marked by a distinct crest, which has been interpreted by the present writer (Hotton, 1959) as an attachment for part of the tympanum. The line of attachment of the tympanum may be called the tympanic crest of the stapes. This arrangement is considered to have arisen in the following manner. During closure of the otic notch, the temporal bones that had supported the tympanum in the amphibian were brought closer to the body and internal process of the stapes and the tympanic process was shortened. By the time the notch was completely closed and could no longer support the tympanum, the former dorsal margin of the membrane had become attached to the internal process, which may have become somewhat larger and more heavily ossified to receive it. The tympanic process was shortened out of existence and its function assumed by the medial attachment of the tympanum to the stapes.

The tympanic crest is interrupted by a small notch or a smooth area at the base of the dorsal process (fig. 3) in three specimens seen by the writer. Two are from a single individual of *Ophiacodon* (MCZ 1366, cast) and one is the fragmentary right stapes of *Clepsydrops* in the Chicago Natural History Museum (UR 329). In the only specimen of *Dimetrodon* at hand (left stapes, MCZ 1347, cast) the area at the base of the dorsal process is chipped, but there can be little doubt that this region was smooth also. The notch is interpreted as the point at which chorda tympani curved forward to cross the stapes (fig. 2, B), assuming that the facial nerve followed the same course in the pelycosaurs that it does in all living tetrapods, and that chorda tympani came off the main trunk of the nerve behind the stapes.

In front of the stapes of *Dimetrodon* and *Ophiacodon* the quadrate is shallowly concave. This concavity does not reach the lateral surface of the skull, being "walled off" by the squamosal which is here interpreted as the lateral attachment of the ear drum. With the tympanum supported between squamosal and stapes, the tympanic cavity must have been restricted to a shallow space anterior to the membrane; it passed medially in front of the stapes to the pharynx. The outer surface of the tympanum faced posteriorly and was reached by a short external auditory meatus that passed behind the squamosal below the tip of the tabular. Since the tympanum was attached to the stapes so that it lay in the same plane as the ossicle, the back of the stapes could not lie within the tympanic cavity. It may have been contiguous with the innermost recess of the external auditory meatus, covered intimately with epithelial lining of the meatus. However, this relationship would be expected to leave impressions of the facial nerve and its branches on the back of the bone. Such impressions do not exist, so it is more likely that the meatus ended at the junction between tympanum and stapes, the

back of the bone being covered loosely by the areolar connective tissue that lay deep to depressor mandibulae and trapezius muscles. Technically, chorda tympani lay partly outside of the tympanic cavity in the large pelycosaurs, only entering the cavity proper as it crossed the shaft of the stapes; it ran a short distance in the medial margin of the tympanum before descending to the quadrate.

Once its nature is understood, this type of reptile middle ear can be recognized even in specimens in which the stapes has been lost, by the lack of an otic notch and by the placement of the quadrate below the level of the otic capsule. This pattern of otic region is also found in such primitive captorhinomorphs as *Melanothyris, Protorothyris,* and probably *Limnoscelis.*

Comparison between anthracosaurian and reptilian middle ears. The obvious differences between the middle ears of primitive reptiles and anthracosaurs, i.e., loss of tympanic process, oblique orientation of stapes, and different placement of tympanum, are all due to closure of the otic notch, the result of changes in the jaw apparatus that only secondarily affected the middle ear. They are characteristic of primitive pelycosaurs and captorhinomorphs, but except for the somewhat lower position of the tympanum are not particularly "reptilian" in nature. Similarities between the middle ears in these forms are equally striking and significant. It is noteworthy, for example, that in very primitive reptiles the chorda tympani was not yet pre-tympanic and the quadrate still lay below the otic capsule as in the anthracosaurs. The new attachment of the tympanum utilized the amphibian internal process and did not require alteration of the position of the stapes or the development of any new structure.

Origin of modern types of middle ear (fig. 2, C). The next stage in middle ear evolution is marked by raising of the quadrate to the level of the otic capsule. Since the internal process remained in contact with the lower end of the quadrate, the stapes lost its oblique orientation and became horizontal; chorda tympani was still draped over the internal process and was pushed up ahead of it as it rose. The tympanum was forced from its attachment on the internal process and came to lie at the distal end of the stapes. The tympanic cavity followed the membrane in its distalward migration and eventually surrounded the stapes completely. At this stage the chorda tympani at last became definitively pre-tympanic, but it could not have reached this position without prior closure of the otic notch.

In the captorhinomorph line the progressive raising of the quadrate is beautifully illustrated by the series *Protorothyris-Romeria-Captorhinus,* as recently restored by Watson (1954). *Protorothyris* is the oldest of the three and *Captorhinus* the youngest, and the series, including as yet unnamed intermediates, may represent an almost completely preserved phyletic line, as Watson suggests. If this interpretation is correct, raising of the quadrate and horizontal placement of the stapes must have taken place quite rapidly in the captorhinomorphs.

The process occurred independently and in somewhat different fashion among the pelycosaurs. These animals show a much greater range of overall size than do the captorhinomorphs, and the advanced type of middle ear, with horizontal stapes, seems to be characteristic of the smaller members of the group. The smaller pelycosaurs, comparable in size to the smaller captorhinomorphs, are usually spoken of as being more primtive, but all of them so far described show enough specialization to be assignable to one or another of the three suborders into which Romer and Price (1940) divide the Pelycosauria. Most of them appear after the earliest appearance of *Clepsydrops* and *Ophiacodon. Clepsydrops* is the oldest known pelycosaur and one of the most primitive morphologically; individuals of its smaller species are two to three times as large as most captorhinomorphs.

Ophiacodon is also very early in appearance and very primitive in structure, but attains a much greater size than does Clepsydrops. If moderately large size is characteristic of the most primitive reptiles, it follows that the smallness of the small pelycosaurs is a specialization in itself. Raising of the quadrate took place in relation to changes in jaw mechanism, and among pelycosaurs the most obvious selective factor that might bring about such a change is change in food habits related to reduction of overall size of the animal. Therefore it is reasonable to claim that however generalized they may be in other respects, the small pelycosaurs show a more advanced condition of the middle ear than do *Ophiacodon* and *Clepsydrops*.

The tympanum maintained contact with the stapes by means of a tag of connective tissue which became chondrified (or, in a few cases, ossified) to form a new tympanic process, one that was not present in primitive pelycosaurs and was not homologous with the tympanic process of amphibians. This neomorphic reptilian tympanic process could have arisen either distal or proximal to the chorda tympani, and the factor that determined its position with respect to the nerve was probably nothing more significant than chance. The left side of fig. 2, C shows the consequences of tympanic process arising distal to the nerve, which looped over the stapes between tympanic and dorsal processes. With these relationships established, all necessary conditions existed for the appearance of the middle ear of diapsid reptiles and birds.

The right side of fig. 2, C represents the alternative possibility, in which the new tympanic process arose proximal to the chorda tympani. This may be the condition from which the mammalian ear was ultimately derived, and is based upon a suggestion of Vaughn (1955) that in the pre-mammalian line chorda tympani did not necessarily loop over the top of the tympanic process. Instead, the nerve became trapped between the tympanic process and the connection between internal process and quadrate (see also fig. 2, E), and eventually was forced into the tympanum upon reduction of the quadrate.

These relationships, which in later and more complex manifestation came to acquire deep phylogenetic significance, may thus be of very simple origin. Their very simplicity makes it possible that they could have arisen either way in closely related forms. On the other hand, they could not have appeared until after the raising of the quadrate, which in turn seems to follow historically the closure of the otic notch. In other words, these distinctive features did not appear until after the tetrapods had become reptiles by any osteological criteria that one may apply.

Function and the Causes of Change

The middle ear originated and evolved as a part of a functioning organism upon which selection was acting at all times. Its chief components were derived from anterior visceral structures that were related to the jaw and respiratory mechanism of fish ancestors, and its early history was closely associated with that of the jaws and back of the skull. Throughout this period two types of change may be recognized. One of them is characterized by modifications of jaw structure which affected the middle ear; in this case the primary selective pressure was toward firmer jaw suspension, perhaps in response to changing environmental conditions, and the effect on the middle ear was secondary. The second type of change consisted of modification of middle ear structure toward more effective detection of fluid-borne sound, which presumably carried a selective advantage under most circumstances. These types of change were probably never entirely independent of each other, but at different times one type contributed more than the other to the general modification of the middle ear.

The fish hyomandibular had evolved as

a support for the jaws. Although its supportive function in the rhipidistians was probably restricted to the operculum it was still primarily adapted to this function. It probably worked in a crude way to transmit water-borne sound that was picked up, equally crudely, by the operculum, but this was only incidental, a result of its long-standing contact distally with the superficial operculum and proximally with the surface of the otic capsule. The spiracular cleft lay between the hyomandibular and braincase as an anterior outlier of the gill system; it was merely another vertebrate characteristic of great antiquity and any contribution it may have made to a sound conducting function was also incidental.

Transition from a rhipidistian to a labyrinthodont type of skull was characterized by a forward migration of the quadrate and loss of the fish operculum. Shift of the quadrate was related to changes in jaw suspension, and loss of the operculum to changes in the axial skeleton as the animals began to develop necks in response to changed habits of food-gathering. These alterations were of primary importance in conversion of the hyomandibular into a stapes, the quadrate shift carrying the ossicle from a fore-and-aft fish orientation to a transverse amphibian orientation, and loss of the operculum freeing the bone from any remnant of suspensory function. All of the structural elements of the amphibian middle ear were present in the fish, and when the operculum disappeared, the sound detecting and conducting functions of the apparatus became dominant. Selection then modified this melange of a displaced suspensor and a tag-end gill pouch toward a specifically sound-conducting functional unit. Final conversion to a middle ear required only a thinning out of the outer wall of the spiracular pouch where the operculum had lain, and probably took place very rapidly. Further changes in the labyrinthodont middle ear were such refinements as perforation of the fenestra ovalis and probable slight reduction in size of the stapes.

The combination of tympanum, stapes, and tympanic cavity that first appeared in the amphibians was a functional unit. The tympanum was set in motion by vibrations of a fluid medium, at first water and later air. The stapes communicated these vibrations to the inner ear, at first via the wall of the otic capsule and later via the perforate fenestra ovalis. The tympanic cavity provided a space in which tympanum and stapes could vibrate with relative freedom. It would therefore tend to be maintained in close relationship to the tympanum and to that axis of the stapes across which the greatest vibration took place, which can be called the functional axis of the stapes. Selection toward maintenance or refinement of this sound-conducting mechanism tended to act upon the middle ear as a whole.

On the other hand, outside forces occasionally tended to alter a single part of the ear, but when this happened selection for effective hearing acted to maintain all parts in a functional relationship. Closure of the otic notch is an example of such an outside force, being directly and exclusively the result of further changes in jaw structure which swung the upper end of the quadrate backward and brought the bone into a vertical reptilian position. It acted primarily upon the tympanum, forcing it downward to gain attachment to the internal process. Meanwhile, selection maintained the middle ear as a functional entity by causing the tympanic cavity to keep pace with the tympanum in its descent. Although some workers believe that middle ear changes from amphibian to reptile changed the mode of sound detection in the latter, it is very probable that the selection that brought the tympano-stapes type of auditory apparatus into existence in the first place was strong enough to maintain it in the face of all documented changes in jaw structure.

In the amphibians the terminal attachment of the tympanum produced a

functional axis in the stapes that ran transversely from tympanic process to footplate and was surrounded by the tympanic cavity. The functional axis did not involve the internal process, which therefore lay outside the cavity. In early reptiles, on the other hand, the low attachment of tympanum to internal process produced a functional axis that ran obliquely from internal process to footplate; the tympanic cavity was still associated with the axis, which meant that it now became involved with the internal process. Since the amphibian tympanic cavity is homologous with the spiracular cleft, its descent (or the descent of an evagination from it) with the descent of the tympanum was probably the phylogenetic origin of the ventral diverticulum seen in amniote embryos.

Whenever the jaw mechanism became stable, or when it ceased to have an effect upon the middle ear, modifications of the auditory apparatus toward improved function became increasingly evident. In most lines this took place after the stapes had become horizontal, but in limited cases it occurred while the stapes was still in its primitively oblique position. In the large sphenacodont pelycosaur *Dimetrodon*, for example, the stapes is strongly flattened in the plane of the tympanum and the articular surface of the dorsal process is apparently part of a diarthrodial joint. These features represent an advance over the more primitive ophiacodont condition, and may have become established following stabilization of the sphenacodont jaw articulation in a primitively low position, when the animals achieved temporary success as large predators during the early Permian.

The peculiar conditions of the diadectid ear, such as the relatively small stapes and concave quadrate, may comprise another example of such an early trend, following jaw stabilization in response to demands of a specific ecologic niche. The diadectid modifications were quite different from those seen in sphenacodonts and proceeded much further, producing a middle ear which resembled that of diapsid reptiles.

However, the position of the diadectids among primitive reptiles remains equivocal. As stated in the first part of this paper, it is doubtful that chorda tympani could have migrated to the position it occupies in modern reptiles without the ancestral otic notch having been closed. If the diadectid otic notch represents a holdover of the amphibian notch, the diadectids could not have given rise to any modern form. If, on the other hand, the diadectids are ancestral to turtles (Olson, 1947) or to all modern reptiles (Watson, 1954) one must search for early forms of diadectid affinities in which the otic notch has been lost and the cheek region of the skull is more nearly captorhinomorph in appearance. *Bolosaurus* fulfills such requirements, but occurs too late in time to provide a link between primitive captorhinomorphs and diadectids. It is not within the scope of this paper to discuss the nature of the middle ear of *Diadectes* in the light of concepts set forth here, but certain features described by Watson suggest a closer similarity to the ophiacodont stapes than he suspected. Despite the close scrutiny to which *Diadectes* has already been subjected, further study of the animal and its relatives holds promise of additional reward.

Later Evolution of the Middle Ear

In the proto-diapsid line, jaw changes affecting the middle ear ceased about the time the stapes became horizontal, and the pattern of middle ear modifications remained fairly simple. The tympanum became larger so that it had to be attached along the whole height of the quadrate (fig. 2, D) instead of only at the top of the bone, and the quadrate (and with it the squamosal) became more concave for reception of the enlarged membrane. The stapes became slimmer and less massive.

Among captorhinomorphs the late lower Permian genus *Captorhinoides* (Olson, 1951) shows a much more slender stapes

than does its immediate predecessor *Captorhinus,* although no excavation of the posterior cheek region is evident in either of these genera. If, as Parrington (1958) argues, the later Permian millerettids are of captorhinomorph derivation, they show a final stage in development of an essentially diapsid type of middle ear, complete with slimmed-down stapes and posteriorly excavated quadrate.

Mammalian conditions and their origin. Changes in the middle ear in the line leading to mammals also include enlargement of the tympanum and reduction of the stapes, which may be interpreted as adaptations for improved sound detection and transmission. But the pattern is complicated by a general reduction of the back of the skull and jaws that continued for a long time in the history of these animals.

Chorda tympani crosses the medial surface of the mammalian ear drum; the part of the membrane that lies above the nerve is called pars flaccida (Shrapnell's membrane) and the part below it pars tensa. Pars flaccida is usually homologized with the reptilian tympanum because of its lack of a middle layer of tissue of mesodermal origin (cf., e.g., Versluys, 1899; Gregory, 1910; Westoll, 1945; Vaughn, 1955). Pars tensa is considered to be a neomorph that appeared with the advent of a generally mammalian level of organization. The presence of elements of lower jaw origin in association with pars tensa is usually held to indicate that it came from a more ventral level than did the original reptilian membrane.

Palmer (1913) observed the close similarity of the tympanic bone in the fetal bandicoot (*Perameles*) to the reflected lamina of the angular bone in late therapsids. Watson (e.g., 1951, 1953) and Westoll (1943, 1945) have concluded from this evidence that a new diverticulum from the tympano-eustachian cavity found its way to the vicinity of the reflected lamina in the later therapsids. The diverticulum did not at first reach the surface to form a definitive tympanum, according to this theory, but persisted for a time as a simple chamber, possible function of which remains obscure; Westoll suggests that it may have formed a vocal resonating chamber. Eventually it formed a new membrane supported on the reflected lamina. Upon shortening of the back of the skull and articular region of the jaw it moved closer to the old reptilian cavity, bringing the new membrane into confluence with the reptilian membrane.

The theory that the mammalian middle ear chamber originated as an evagination of the reptilian cavity consists mainly of a description of the sequence of events in mammalian ontogeny fitted into a phylogenetic frame of reference. The selective forces that would induce such an evagination are obscure. I suggest that there was never more than one membrane, and that it arrived at the mammalian infra-mandibular position simply because it got larger at the same time the quadrate was getting smaller. Increase in tympanum size was the result of selection toward more effective middle ear apparatus as it was in the protodiapsids, while reduction of the quadrate was related to changes in jaw structure.

At first the membrane lay behind the quadrate as it did in the ancestors of diapsid reptiles, and as in those forms it expanded downward toward the jaw articulation. But reduction of the quadrate in later synapsids tended to restrict the space available for expansion of the membrane. Perhaps the insertion of the depressor mandibulae muscle prevented the drum from expanding below the articular bone for a time. Eventually, however, reduction of the posterior jaw elements and associated structures permitted the membrane to extend below the jaw articulation. Shortening of the back of the jaws and skull brought the angular, with its reflected lamina, closer to the point at which the tympanum would reach below the lower jaw, so that when the membrane finally got there it would fall right into the arms of the angular, so to speak. The

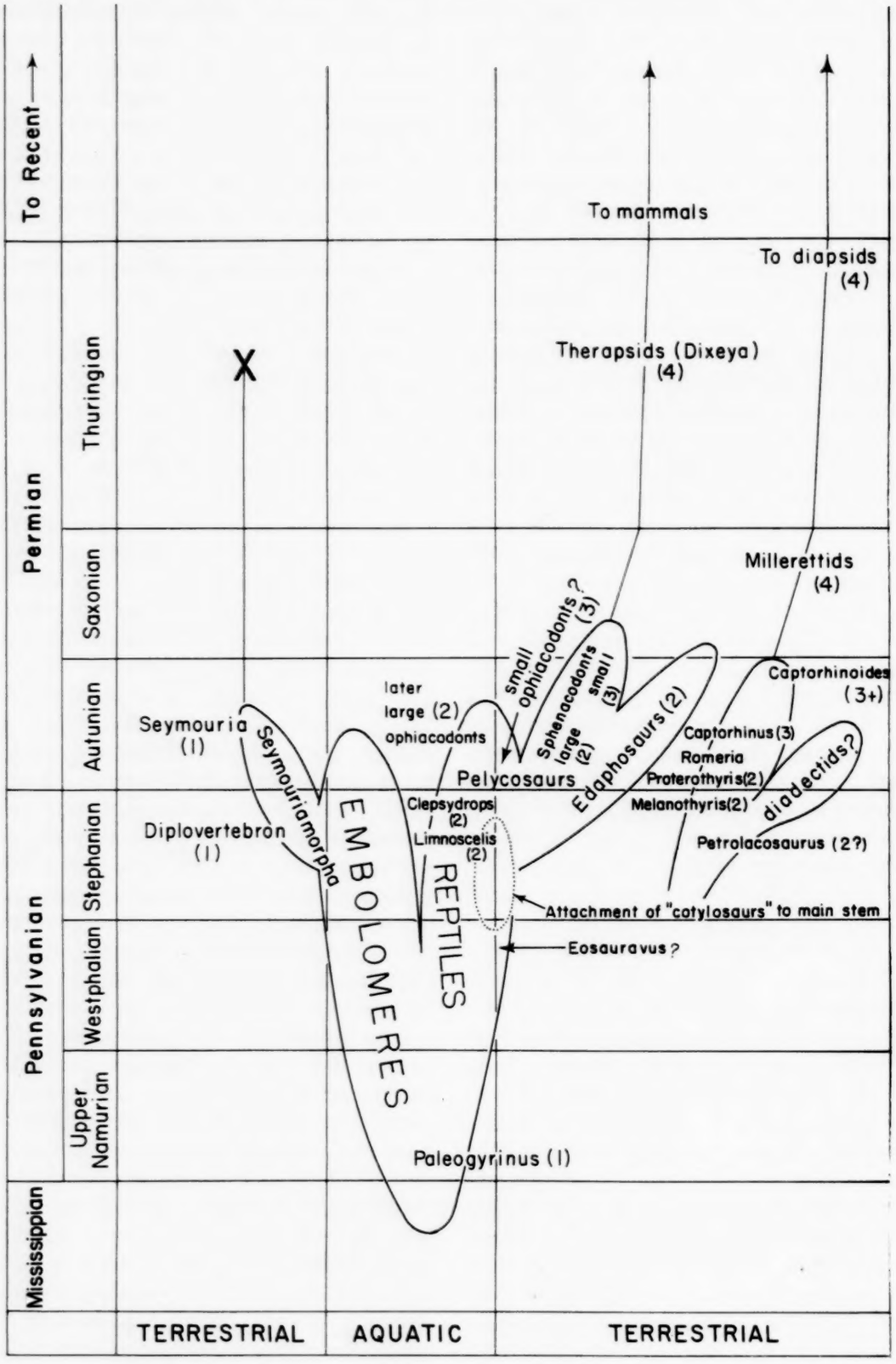

FIG. 4. Possible phylogenetic relationships of late Paleozoic anthracosaurs and reptiles, as controlled by radiation into terrestrial environments. Numbers enclosed in parentheses indicate stages of middle ear evolution as follows: (1) otic notch and amphibian tympanic

close functional and ontogenetic association between tympanum and tympanic cavity would insure that the cavity would follow the membrane faithfully in this phylogenetic migration. In late stages the low position of the tympanum would come to require a sufficiently prominent outpouching of the embryonic reptilian cavity to produce the phenomena observed in mammalian ontogeny.

The writer recently had the opportunity to examine the beautifully-prepared skull of a Triassic cyodont from Ischigualasto, Argentina, and to discuss it with its describer, Professor Bryan Patterson of the Museum of Comparative Zoology, Harvard. The specimen is complete and virtually undisturbed in the otic region. It is quite evident that a large functional membrane must have been present, supported dorsally by the squamosal (representing the old reptilian attachment) and antero-ventrally by a ventrally-directed process of one of the small bones of the lower jaw (the coming "mammalian" attachment). Thus it demonstrates clearly an intermediate stage in the descent of a single tympanum from reptilian to mammalian position.

Professor Patterson showed that the only possible identification of the bone with the process on it is pre-articular, not angular. The angular is present and recognizable, but is perfectly smooth and has lost its reflected lamina. This fact, coupled with the general lack of a reflected lamina in late cynodonts, casts doubt on the long-accepted homology of the mammalian tympanic bone with the reptilian reflected lamina. The embryological evidence for this homology is not unequivocal, and should be reviewed in the light of more recent paleontological evidence.

However, the exact homology of the lower jaw element supporting the tympanum in mammals and advanced cynodonts is not relevant to the present discussion. The Harvard specimen demonstrates that final transition of jaw elements into middle ear ossicles must have taken place after the tympanum had gained support on the lower jaw precursor of the tympanic bone. The transition probably occurred as the membrane relinquished its reptilian attachment to skull elements, when the squamoso-dentary jaw articulation was finally established. Although transition of the membrane from partial post-quadrate support to complete support by the tympanic bone must have been quite rapid, it was probably continuous and should not be thought of as saltatory in nature; there is no need to postulate a non-functional phase during transition from reptilian to mammalian position.

Conclusions

The middle ear appears to have gone through four main stages in its evolution. The first stage is that of the labyrinthodont, which is characterized by an otic notch and a tympanic cavity homologous with the spiracular cleft; chorda tympani is post-tympanic in position. The second stage is found in large and primitive pelycosaurs and the most primitive captorhinomorphs, and is characterized by loss of the otic notch, an obliquely-oriented stapes, and attachment of a large part of the tympanum to the internal process; chorda tympani does not yet lie in the pre-tympanic position. The third stage is that shown by small pelycosaurs and advanced captorhinomorphs, and is characterized by a horizontally-oriented stapes with tympanum lying terminal to it and a neomorphic tympanic process; chorda tympani has finally come into a pre-tympanic position. Two divergent fourth stages are shown by the lines lead-

process present; (2) no otic notch, tympanum supported in part by internal process, massive stapes oriented obliquely; (3) no otic notch, tympanum terminal to horizontal stapes, new reptilian tympanic process appears; (4) tympanum terminal to stapes, stapes reduced, quadrate concave (pre-diapsid) or reduced with evidence of external auditory meatus directed toward it (pre-mammal).

ing to diapsid reptiles and to mammals, respectively. Both are characterized by modifications for improved detection of air-borne sound; they differ in origin only because of fortuitous differences in the relationship between the new tympanic process and chorda tympani, and they diverge because of a continuation of jaw changes that affected the middle ear in the line leading to mammals.

Figure 4 represents the stratigraphic occurrence of the forms discussed in this paper, and shows the temporal sequence of morphological stages of the middle ear. *Seymouria, Diplovertebron, Eosauravus,* and *Petrolacosaurus* are also included in figure 4; they are genera pertinent to a consideration of reptile phylogeny that have not been discussed in preceding pages. *Seymouria* is a central genus of the suborder Seymouriamorpha, a line of anthracosaurs which is characterized by an extremely reptile-like postcranium but persistently amphibian dermal bone pattern and otic notch. *Diplovertebron* is a small upper Pennsylvanian anthracosaur which is almost too primitive to be a seymouriamorph but nevertheless shows some terrestrial adaptation. Watson (1926) pointed out the terrestrial features of *Diplovertebron* and compared it to the rhachitome *Cacops*. Many of the reptilian features of Seymouria also resulted from adaptation to terrestrial habits, and it is possible that the Seymouriamorpha represent a development among anthracosaurs comparable to the dissorophids among the rhachitomes.

Eosauravus is a small, terrestrially adapted animal of the middle Pennsylvania which has been called a seymouriamorph, but which most recently (Peabody, 1959) has been referred to the captorhinomorph reptiles on the basis of tarsal structure. The anterior part of the animal is not known. If Peabody is correct, *Eosauravus* is the oldest known reptile, and antedates the first appearance of the Seymouriamorpha by one-third of the Pennsylvanian period. *Petrolacosaurus* (Peabody, 1952) is another small terrestrial form that appears to be referable to the captorhinomorphs. According to Watson (1954) its otic region is similar to that of primitive captorhinomorphs and pelycosaurs. Although it shows specializations in skull fenestration and elongation of neck and limbs, it antedates the other captorhinomorphs and earliest pelycosaurs.

The provisional phylogenetic pattern shown in figure 4 is based chiefly upon a suggestion of Romer (1946) that the morphological indications of an aquatic habit shown by *Limnoscelis* and the early ophiacodonts, which were relatively large forms, are holdovers from their amphibian ancestry and not secondary adaptations. This interpretation implies that these animals were derived directly from a large equatic embolomere like *Paleogyrinus* without an intervening terrestrial phase of adaptation; the pelycosaurs and their embolomerous ancestors then constitute the main trend of tetrapod evolution during Pennsylvanian time. Comparing *Paleogyrinus, Limnoscelis,* and the ophiacodonts with *Diplovertebron, Eosauravus,* and the early captorhinomorphs, one is struck by the apparent correlation of small size with terrestrial adaptation among Pennsylvanian tetrapods, irrespective of their formal taxonomic disposition. These small forms represent successive experiments in terrestrial specialization produced by the main line of tetrapods. *Diplovertebron* came from a conservative member of the main line at an amphibian level of organization and probably represents the origin of the Seymouriamorpha; *Eosauravus* arose after attainment of a reptilian level and perhaps gave rise to the captorhinomorphs, most of which are small. The many similarities between seymouriamorphs and captorhinomorphs are the result of parallel adaptation to terrestrial environment.

The conservative aspect of this phylogeny is largely the result of concepts of middle ear evolution developed in this paper. First, in spite of changes induced by otic notch closure, the middle ear of

primitive reptiles retains a basic similarity to that of anthracosaur amphibians; this strongly supports the theory of anthracosaur ancestry of reptiles. Second, the chorda tympani could not have found its way to the pre-tympanic position it occupies in modern reptiles and in mammals until the otic notch had closed and the stapes had become horizontal. The nerve began its migration, however, with closure of the otic notch, which marked attainment of a technically reptilian level of organization in this line.

The captorhinomorphs and pelycosaurs are distinct as far back as they can be traced, to the upper Pennsylvanian. There is strong evidence that the stapes became horizontal independently in the two groups; the stapes becoming horizontal caused formation of the new reptilian tympanic process, which therefore must have been acquired independently by each group. Placement of the new tympanic process with respect to chorda tympani coincided with this event; the process may have arisen lateral to the nerve in the captorhinomorphs and medial to it in the pelycosaurs. Thus middle ear differences between the line leading to modern reptiles and that leading to mammals could have originated as part of the divergence between the captorhinomorph and pelycosaur reptiles in pre-Permian times.

Evolution of the middle ear was adaptive in that maintenance and refinement of a tympano-stapes type of hearing mechanism carried a selective advantage under most circumstances. Structural modification toward more effective function appeared in all lines whenever jaw stabilization took place. In some lines (e.g., sphenacodonts, diadectids) such modifications occurred very early, but in most they did not become pronounced before the middle Permian.

Goodrich (1930) maintained that tetrapods above the level of amphibians could be divided into two lines of completely independent origin on the basis of the circulatory system and certain characters in the foot. He brigaded nearly all Paleozoic reptiles and mammal-like reptiles together as Reptilia Theropsida, and the diadectids and all other reptiles together as Reptilia Sauropsida. The circulatory system is not a reliable criterion for determining relationship among fossil reptiles, and more recently Watson (1954) has claimed that the two groups can be distinguished on the basis of middle ear structure, the massive stapes and straight posterior skull margin being characteristic of the Theropsida and the slender stapes and concave skull margin being characteristic of the Sauropsida. Most proponents of this system consider the captorhinomorphs to contain the ancestors of the pelycosaurs. Reviews of recent work on the concept are to be found in Vaughn (1955), who favors it, and Parrington (1959), who does not.

The interpretation of the middle ear advocated in the present paper runs counter to the Goodrich-Watson hypothesis, and is supported by the temporal relationships of most of the animals involved. The massive stapes is nearly universal among the earliest reptiles, while the so-called sauropsid type of ear is a later development in all cases except that of *Diadectes*. The massive stapes is merely another of numerous primitive characters possessed by the earliest reptiles and indicates only their proximity to a common source. The slender stapes and excavated quadrate is very probably adaptive in nature, and the selective advantage it carries brought about its appearance in all lines as soon as circumstances permitted, including such early experiments as *Diadectes*. In making a taxonomic distinction between forms with massive stapes and forms with slender stapes and concave quadrate, Watson's theory separates ancestor from descendant, not divergent lines from each other.

There is little good evidence that the captorhinomorphs actually antedate the pelycosaurs. Instead, the earliest representatives of both groups appear to be

contemporaneous, and on the basis of non-otic criteria they appear to be separate developments from a common source. The distinctiveness of captorhinomorph and pelycosaur lines is further supported by the probability that the stapes became horizontal and the chorda tympani became pre-tympanic independently in the two groups. Herein lies the true separation of reptiles into two lines, one leading to diapsid reptiles and birds, and the other to mammals. But both lines were derived from forms in which the otic notch was closed, animals which were, in other words, technically reptiles. There is at present no evidence to indicate whether or not closure took place independently in these two lines, nor is the question relevant. Traced backward, the captorhinomorphs and pelycosaurs show a high degree of convergence; their latest common ancestor must have been as much a reptile as they, for all we can tell, whether it had an otic notch or not.

Thus, if the terms Reptilia Theropsida and Reptilia Sauropsida are to be retained, they must be redefined in such a way that the Pelycosauria are included in the former and the Captorhinomorpha in the latter. But the concept behind the terms implies a much greater difference than that which obtains between the two Permo-Carboniferous groups, and this degree of difference is not apparent morphologically in their descendants until the middle Permian. It is therefore undesirable to use them to distinguish between Captorhinomorpha and Pelycosauria, and since adequate taxonomic nomenclature already exists to separate later reptilian lines, the terms Reptilia Theropsida and Reptilia Sauropsida should be dropped.

The origin of the diadectids and the question of whether or not they gave rise to any post-Paleozoic forms are beyond the scope of this paper. Contrary to the relationships shown in figure 4, the diadectids may have been derived from seymouriamorph anthracosaurs, and they may be ancestral to turtles as suggested by Olson (1947). But the turtle chorda tympani is pre-tympanic, which means that if the diadectids did give rise to turtles, the amphibian otic notch must have been closed before the definitive diadectid condition was attained.

Acknowledgment

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THE GENETICS OF HYBRID STERILITY IN *DROSOPHILA PAULISTORUM*[1]

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The willistoni species group includes five known sibling species, one of which is *Drosophila paulistorum* Dobzhansky and Pavan. The morphological characteristics and the relationship of this species to *D. tropicalis* Burla and Da Cunha, to *D. willistoni* Sturtevant, and to *D. equinoxialis* Dobzhansky were analyzed by Burla *et al.* (1949). Later, a fifth sibling species, *Drosophila insularis* Dobzhansky, was added to the group (Dobzhansky, Ehrman, and Pavlovsky, 1957). The five sibling species can be told apart by crossability tests and by inspection of the chromosomes in the cells of the larval salivary glands; moreover, Spassky (1957) found minute differences in the structure of the genitalia, which permit him to classify living males into the five species categories. Without doubt, the sibling species are completely isolated reproductively. Although interspecific insemination does occasionally occur in laboratory experiments, no viable hybrids are produced except in crosses involving *D. insularis,* where the hybrids are wholly sterile. This species, *D. insularis,* is most restricted in its geographic distribution. It is known only from two islands in the Lesser Antilles, while the other four species are widespread over the American tropics. *D. tropicalis, D. willistoni, D. equinoxialis,* and *D. paulistorum* are sympatric in a region including the Amazonian basin and the part of South America that lies north of the Equator.

D. willistoni has been found to be a unified species—one not divided into subspecies showing any trace of incipient reproductive isolation. *D. tropicalis* includes two known subspecies which produce sterile male and fertile female hybrids (Townsend, 1954). *D. equinoxialis* has not yet been studied. Extensive collections of *D. paulistorum* from localities in Central America, the Caribbean, and South America yielded a group of geographic strains which, when brought into the laboratory and intercrossed, gave varied, and surprising, results. Not all the strains could be mated with equal ease; some matings produced no hybrids at all, while others yielded fertile female hybrids and F_1 males that were completely sterile (Dobzhansky and Spassky, 1959).

Thus, the species *Drosophila paulistorum* represents remarkably favorable material for the study of the genetics of incipient reproductive isolation. It is distributed over a vast area, it has only three pairs of chromosomes, and above all, it offers a number of the very interesting race-species "borderline cases" for analysis. We have here a series of intergrading subspecies, such that when the extremes of this series come together the reproductive isolation between them is virtually complete. Yet, gene exchange via geographically intermediate populations is possible, although it is an open question how much exchange actually does occur. The purpose of the present work is to analyze the *D. paulistorum* complex of nascent species with regard to the causation of the hybrid male sterility observed in crosses between the subspecies.

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Material

As a result of systematic intercrosses of the strains available, six groups of geographic populations were distinguished by Dobzhansky and Spassky (1959), namely:

1. the *Centro-American* group, Guatemala to Costa Rica
2. the *Amazonian* group, Panama through northern Brazil
3. the *Andean-South Brazilian* group, southern Colombia, Ecuador, Peru, Bolivia, and southern Brazil
4. the *Orinocan* group, Panama, eastern Colombia, Venezuela, and British Guiana
5. the *Guianan* group, Georgetown in British Guiana (a single strain)
6. the *Transitional* group, several localities in Colombia.

Crosses between the Centro-American, the Amazonian, and the Andean-South Brazilian groups always result in fertile female hybrids and sterile male hybrids. Some of these crosses, however, can be made only with difficulty, owing to strong sexual isolation existing between the strains involved. The present report is concerned with the genetic basis of the sterility of the hybrid males coming from the crosses between the Centro-American and the Amazonian strains, and between the Centro-American and the Andean-South Brazilian strains.

For the purposes of this investigation, it was necessary to have each pair of chromosomes in the subspecies crossed marked by at least one convenient mutant gene. Once a sufficient number of mutant genes had been acquired chiefly via exposure to X-rays, and these mutations firmly established in stocks, their positions on the chromosomes were mapped (Malogolowkin and Ehrman, in this issue). (The crosses between the Amazonian and the South Brazilian strains, which also yield sterile male hybrids, will be studied by Dr. C. Malogolowkin).

The Experimental Procedure

Figure 1 shows the plan followed in ascertaining the role of the autosomes in the production of the sterility in the hybrid males. The object was to transfer one marked chromosome into the nuclear and cytoplasmic background of an alien subspecies. In each generation, equal numbers of males carrying the marker gene and of wild type sons were dissected in physiological saline and checked for the presence of motile spermatozoa. Note that, at no time could two marked chromosomes be transferred, across subspecific boundaries, simultaneously. *D. paulistorum* is a species delicate enough, so that, at least in laboratory cultures, flies carrying several mutant genes are apt to show a viability too low to permit experiments of this sort to be carried out successfully.

The control experiments involved crosses using females which were identical with the experimental backcross females except for the fact that they did not carry the one marked chromosome. This, as the results will show, further emphasizes the role of single chromosomes in the normal development of male gonads.

Because of the extreme difficulty in succeeding with inter-subspecific matings, the hybrid progeny necessarily come from a limited number of females and the initial inter-subspecific crosses were made in a direction which minimized the sexual isolation barrier. Thus, figure 2 indicates that the Amazonian × Andean-South Brazilian cross was made using Amazonian (marked) males, and Andean-South Brazilian (wild type) females, whereas the Amazonian × Centro-American mating could *only* be accomplished by using Amazonian (marked) females, and Centro-American (wild type) males. Sex-linked marker genes are, as shown in figure 2, very scarce in this material. Only one such mutant was found to be suitable for the experiments, namely a

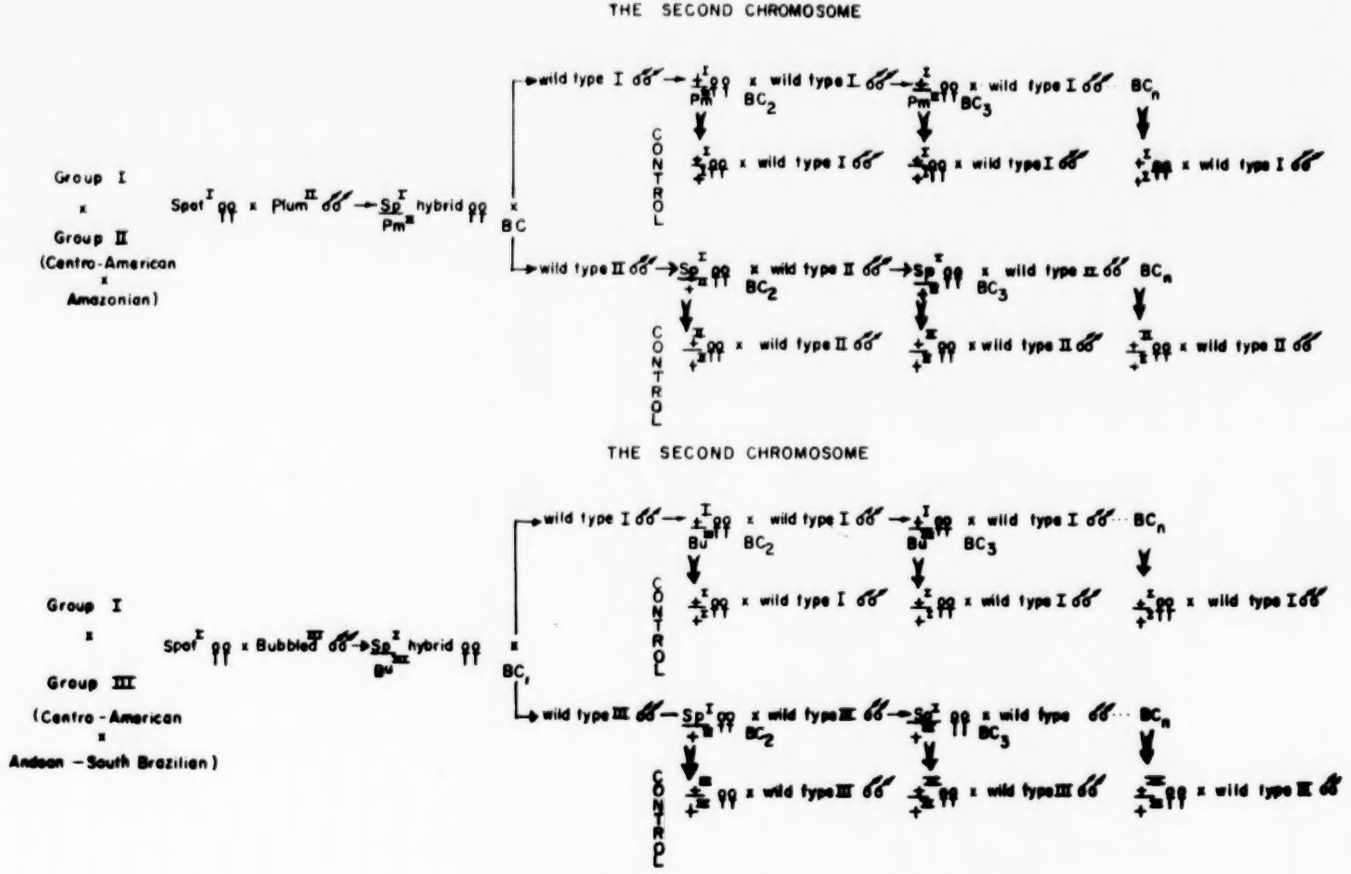
THE SECOND CHROMOSOME
Group I
x
Group II
(Centro-American
x
Amazonian)
Spot I ♀♀ x Plum II ♂♂ → Sp I / Pm II hybrid ♀♀
x
BC
wild type I ♂♂ → I/+ ♀♀ x wild type I ♂♂ → I/Pm II ♀♀ x wild type I ♂♂ BC_n
BC2
BC3
CONTROL
I/+ ♀♀ x wild type I ♂♂
I/Pm II ♀♀ x wild type I ♂♂
I/+ ♀♀ x wild type I ♂♂
wild type II ♂♂ → Sp II/+ ♀♀ x wild type II ♂♂ → Sp II/+ ♀♀ x wild type II ♂♂ BC_n
BC2
BC3
CONTROL
II/+ ♀♀ x wild type II ♂♂
II/+ ♀♀ x wild type II ♂♂
II/+ ♀♀ x wild type II ♂♂
THE SECOND CHROMOSOME
Group I
x
Group III
(Centro-American
x
Andean-South Brazilian)
Spot I ♀♀ x Bubbled III ♂♂ → Sp I / Bu III hybrid ♀♀
x
BC1
wild type I ♂♂ → I/+ ♀♀ x wild type I ♂♂ → I/Bu III ♀♀ x wild type I ♂♂ BC_n
BC2
BC3
CONTROL
I/+ ♀♀ x wild type I ♂♂
I/Bu III ♀♀ x wild type I ♂♂
I/+ ♀♀ x wild type I ♂♂
wild type III ♂♂ → Sp I/+ ♀♀ x wild type III ♂♂ → Sp I/+ ♀♀ x wild type ♂♂ BC_n
BC2
BC3
CONTROL
III/+ ♀♀ x wild type III ♂♂
III/+ ♀♀ x wild type III ♂♂
III/+ ♀♀ x wild type III ♂♂

FIG. 1. Crosses incorporating marked autosomes.

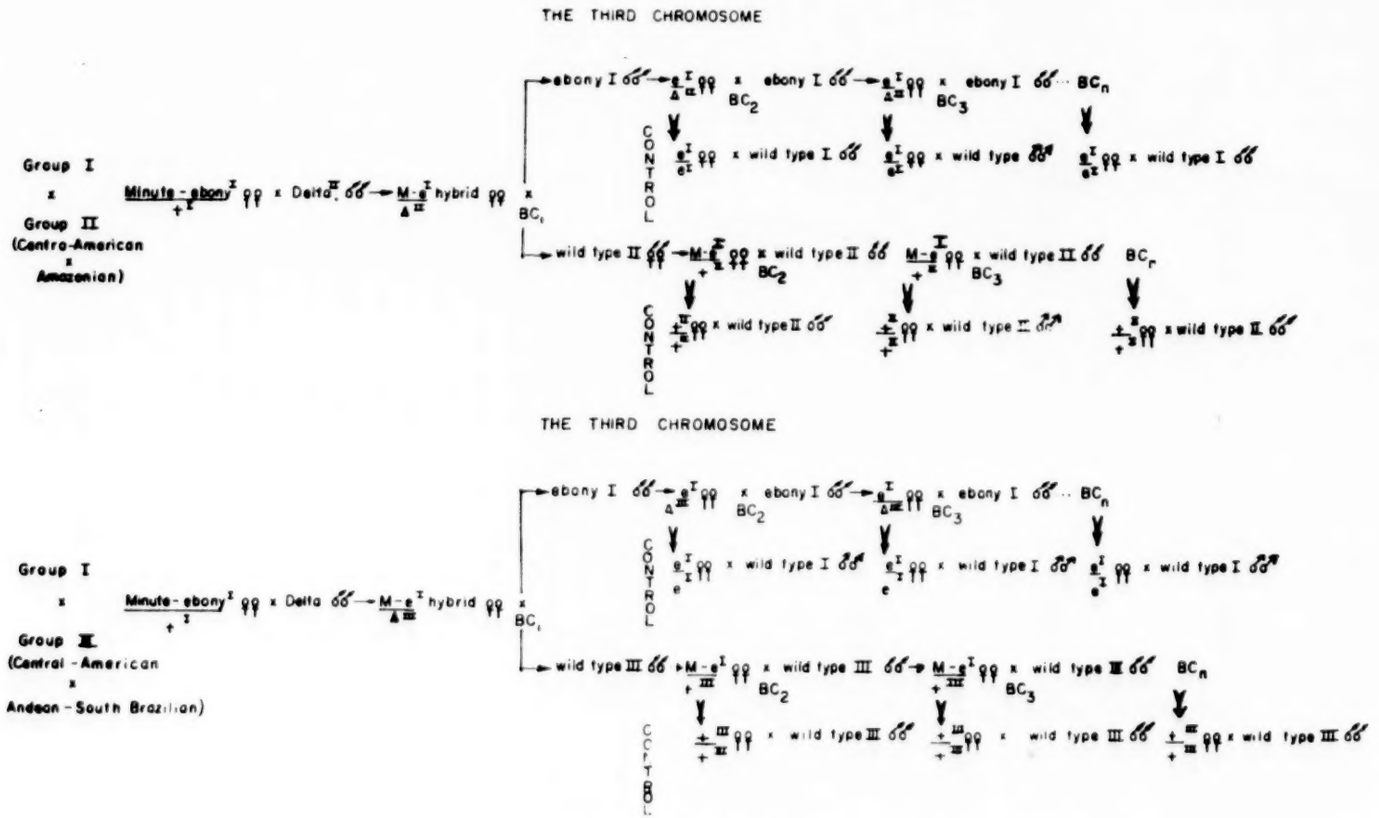

FIG. 1 (continued).

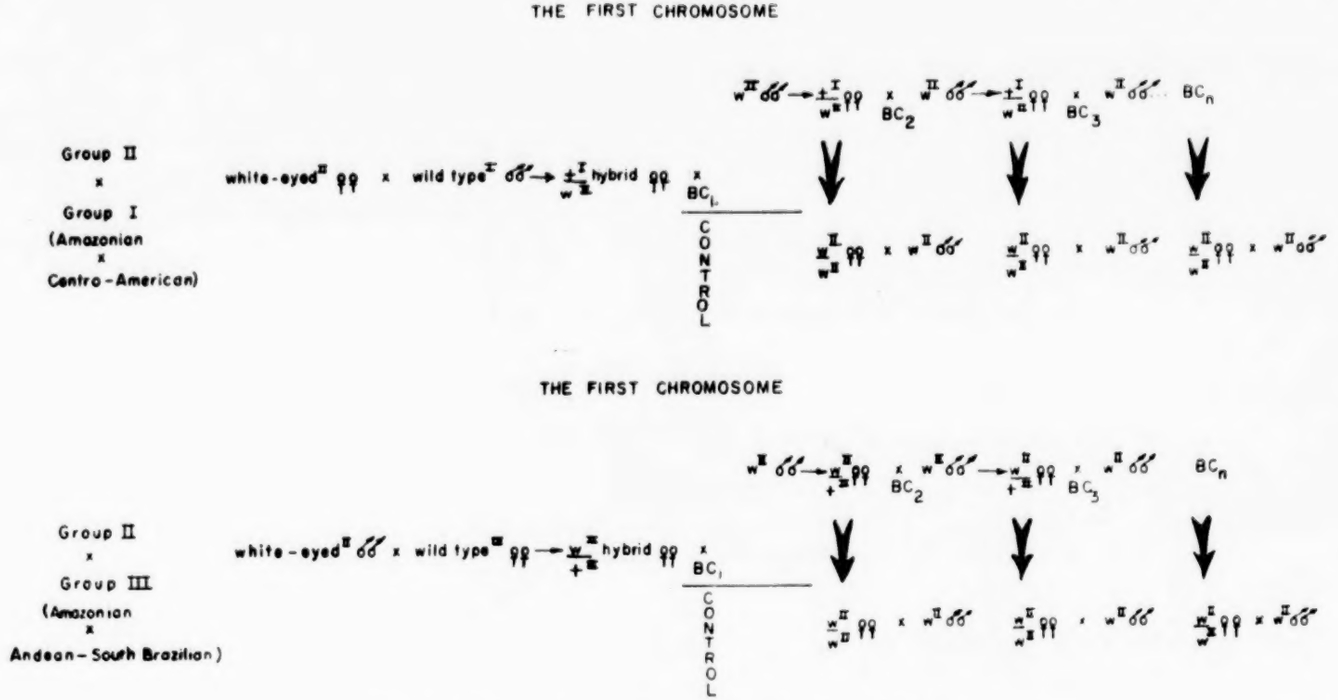

FIG. 2. Crosses incorporating a sex-linked marker.

TABLE 1. *Fractional and percentage dilution of alien chromosome in repeated backcrosses between subspecies "A" and subspecies "B," where subspecies "B" is the recurrent parent*

	Marked chromosomes		Unmarked chromosomes		% "A" in entire genome	% "B" in entire genome
	A	B	A	B		
F_1	1/2	1/2	1/2	1/2	50.0	50.0
BC_1	1/2	1/2	1/4	3/4	33.3	66.7
BC_2	1/2	1/2	1/8	7/8	25.0	75.0
BC_3	1/2	1/2	1/16	15/16	20.8	79.2
BC_n	1/2	1/2	$1/2^{n+1}$	$1 - (1/2^{n+1})$	$(0.5 + 1/2^n)/3$	$1 - [(0.5 + 1/2^n)/3]$
			Controls			
BC_2	0	1	1/8	7/8	8.3	91.7
BC_3	0	1	1/16	15/16	4.2	95.8
BC_n	0	1	$1/2^{n+1}$	$1 - (1/2^{n+1})$	$(1/2^n)/3$	$1 - [(1/2^n)/3]$

white-eyed recessive obtained in the Amazonian group.

Any data from the crosses outlined in figures 1 and 2 would be ambiguous were it not that crossing-over is suppressed in the hybrids, where it occurs, as far as can be detected with all of the markers available, only in negligible quantities. Therefore, though many markers are desirable, and more than one was used wherever possible, one marker per chromosome is enough to distinguish between the presence or absence of that particular chromosome. Notice that each of these crosses provide information about the degree of crossing-over in the hybrids. This is especially true of the crosses involving the marked third chromosomes. Here, the genes *Minute* and *ebony* are fifty crossover units apart in intra-subspecific (Centro-American) crosses, but no crossing-over occurred between them in the hundreds of hybrids examined which carried these marker genes.

In table 1 the degree of hybridity is defined more closely in terms of fractions and percentages. The formulae derived in the table necessarily represent only the theoretically average genome in each generation. (Furthermore, they are inaccurate in that they do not consider the small amount of crossing-over that may occur.) Every inter-subspecific cross was designed to branch out, at the first backcross, into two separate lines, one for each recurrent parental subspecies. Thus, as the backcrosses proceed, the percentage contribution of the recurrent parent keeps increasing until it forms the

TABLE 2. *Results of the crosses outlined in figures 1 and 2, testing the effects of the second chromosome. The figures show the numbers of males dissected in physiological saline, and examined for the presence of motile spermatozoa. Before dissection, the males were tested for fertility. Equal numbers of males carrying the mutant marker and free from it were dissected. S = sterile, no motile sperm; M = sterile, but sperm motile; F = fertile, sperm motile*

Subspecies crossed		Centro-American × South Brazilian	Centro-American × Amazonian
Recurrent parent		Centro-American / South Brazilian	Centro-American / Amazonian
Sons of females with a foreign chromosome	F_1	All S / All S	All S / All S
	BC_1	100 S / 100 S	100 S / 100 S
	BC_2	96 S, 4 M / 95 S, 5 M	95 S, 5 M / 99 S, 1 M
	BC_3	94 S, 6 M / 95 S, 5 M	94 S, 6 M / 92 S, 8 M
Sons of control females	BC_2	9 S, 41 F / 19 S, 31 F	15 S, 35 F / 20 S, 30 F
	BC_3	7 S, 43 F / 11 S, 39 F	5 S, 45 F / 19 S, 31 F

TABLE 3. *Results of the crosses outlined in figures 1 and 2, testing the effects of the third chromosome. For further explanation see the legend for table (2)*

Subspecies crossed		Centro-American × South Brazilian		Centro-American × Amazonian	
Recurrent parent		Centro-American	South Brazilian	Centro-American	Amazonian
Sons of females with a foreign chromosome	F_1	All S	All S	All S	All S
	BC_1	99 S, 1 M	100 S	100 S	100 S
	BC_2	99 S, 1 M	98 S, 2 M	93 S, 7 M	98 S, 2 M
	BC_3	100 S	99 S, 1 M	94 S, 6 M	91 S, 9 M
Sons of control females	BC_2	23 S, 27 F	7 S, 43 F	10 S, 40 F	15 S, 35 F
	BC_3	10 S, 40 F	11 S, 49 F	50 F	16 S, 34 F

greater part of, although never the entire, genome.

We have then a controlled experimental scheme which tests two possible factors:

1. the influence of the genotype of the mother on the fertility of her sons
2. the influence of the genotype of the males on their own fertility.

THE HYBRID MALES

The results can be stated very simply: the sons of hybrid mothers are always sterile regardless of their own genotype. Furthermore, the mothers need only be "hybrid" with respect to one of their chromosome pairs to yield sterile male progeny.

Tables 2, 3, and 4 summarize the data. The F_1 hybrid males are always sterile, and, of course, all of them have the two chromosomes of each autosomal pair derived from the different subspecies crossed. But already in the first backcross (BC_1) some of the males must carry the chromosomal complement of the subspecies to which their fathers belonged. Nevertheless, none of the 1,000 such males dissected had motile spermatozoa, and none of the much larger number tested proved fertile.

The situation changes in the second backcross (BC_2). This backcross can be made in two different ways—utilizing as mothers, females which do and those which do not carry the genetic markers which indicate the presence of the subspecies-foreign chromosome. The former produce again only sterile sons; the latter (control) produce some sterile, but mainly fertile sons. The third backcross is no different from the second. Again the sons of females which carry any foreign chromosome are all sterile, while those of control females are mostly fertile. The question which may arise in a reader's

TABLE 4. *Results of the crosses outlined in figures 1 and 2, testing the effects of the X-chromosome. For further explanation see the legend for table 2*

Subspecies crossed		Amazonian × Centro-American	Amazonian × South Brazilian
Recurrent parent		Amazonian	Amazonian
Sons of females with a foreign chromosome	F_1	All S	All S
	BC_1	94 S, 6 M	93 S, 7 M
	BC_2	94 S, 6 M	94 S, 6 M
	BC_3	93 S, 7 M	99 S, 1 M
Sons of control females	BC_2	5 S, 45 F	12 S, 38 F
	BC_3	6 S, 44 F	10 S, 40 F

mind is, why are not all males produced in the control crosses fully fertile? The answer is simple—in the experiments there was always only a single chromosome, the distribution of which was followed by a genetic marker. When the marker showed the presence of a subspecies-foreign chromosome in a female, the female was assuredly a "hybrid" one. But when the marker was absent, a "control" female may or may not have carried a foreign chromosome, the distribution of which could not be followed because of the absence of a marker for that particular chromosome in a given cross. In other words, among the "controls" there were probably included some hybrid females as well, the male progeny of which had to be sterile.

Dissections have shown that the sons of the hybrid females obtained in the backcrosses have testes as defective or more defective than do the F_1 hybrid males. These latter are, of course, produced by mothers who are not hybrid themselves. It should nevertheless be noted that, particularly beginning with the second backcross, some males had in their testes sperms which appeared to be motile when released in a drop of physiological saline on a slide. And yet these sperms were unable to function in fertilization, as shown by the fact that the females with which these males were kept failed to produce any progeny. These males are designated in tables 2–4 by the letter "M," which stands for motility, not for fertility.

Figure 3 tells the story of a very lengthy series of backcrosses in which a marked autosome was selected for each generation to prevent its loss by replacement. When the sixth backcross was reached, the alien chromosome represented only about one-fifth of the average genome, and the females carrying this autosome were tested for their ability to produce fertile sons. At the same time, their unmarked sisters were similarly tested as controls. The difference in results is quite sharp—all of the sons of

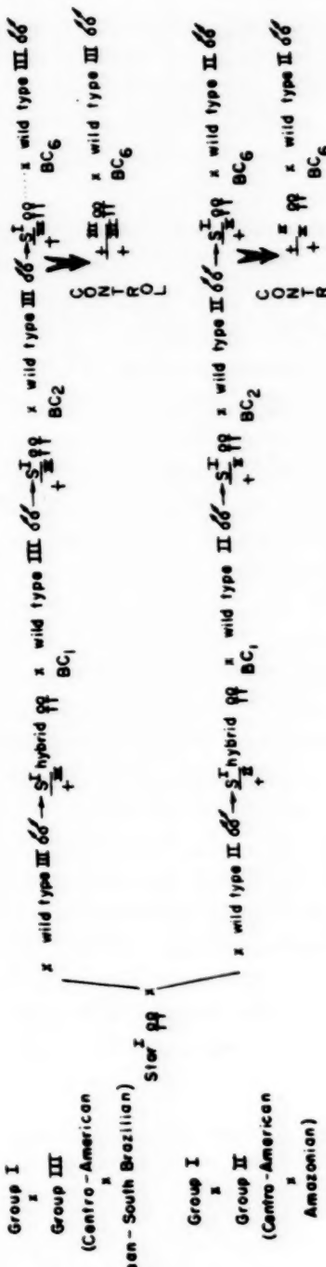

FIG. 3. A special cross, involving the Centro-American second chromosome marker "Star," which was carried to the sixth backcross generation.

TABLE 5. *Results of crosses outlined in figure 3. The figures show the numbers of males dissected in physiological saline, and examined for the presence of motile spermatozoa. Before dissection, the males were tested for fertility. Equal numbers of males carrying the mutant marker (Star) and free from it were dissected. S = sterile, no motile sperm; M = sterile, but sperm motile; F = fertile, sperm motile*

Dissection of sons of:		Experimental	Dissection of sons of:		Control
$BC_6 \frac{S^I}{+^{II}}$	Females	82 S, 18 M	$BC_6 \frac{+^{II}}{+^{II}}$	Females	5 S, 45 F
$BC_6 \frac{S^I}{+^{III}}$	Females	80 S, 20 M	$BC_6 \frac{+^{III}}{+^{III}}$	Females	6 S, 44 F

the "Star" females were sterile, whereas most of the sons of the control females were fertile. In this backcross generation, the control females have virtually no alien chromosomal material, theoretically only 0.5% of their genome, and they are quite effective as mothers.

The principal conclusion which follows from the evidence summarized in table 5 is that the sterility of the backcross males depends upon the genotypes of their mothers and not upon their own genotypes. Females which carry at least one foreign chromosome produce sons all of whom are sterile, despite the fact that some of them must contain chromosomes of one race only and, thus, should be similar to non-hybrid males. On the other hand, in the same backcrosses, mothers which did not carry a subspecies-foreign chromosome produced sons all of whom were fertile. The rule just stated does not apply to F_1 hybrid males, which are sterile despite the fact that their mothers do not carry foreign chromosomes. Presumably the genic imbalance in a hybrid female in some way impairs the cytoplasm contained in her eggs, so that the eggs fertilized by Y-bearing sperms develop into sterile imagos, while those fertilized by X-bearing sperms are consistently fertile as adult females.

Some attempts have been made to explore, in a preliminary way, the disturbances in the testes of the sons of hybrid females which cause the sterility. Testes of the F_1 hybrids and of backcross males were dissected in aceto-orcein and squashed under coverslips. Among the primary spermatocytes, many appeared to show a normal chromosomal pairing, since they contained three bivalents (two metacentric and one acrocentric). Other spermatocytes exhibited some lack of pairing, up to four univalents and one bivalent. No disturbance could be detected, at least with the method employed, in the early part of the first meiotic division. Nevertheless, late anaphases and

TABLE 5A. *Fractional and percentage dilution of the "Star" second chromosome in the sixth backcross generation. (See table 1 for formulae)*

	Chromosome two		Other chromosomes		% Group I in the entire genome	% Group II or III in the entire genome
	Group I	Group II or III	Group I	Group II or III		
BC_6	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{128}$	$\frac{127}{128}$	17.2	82.8
			Controls			
BC_6	0	1	$\frac{1}{128}$	$\frac{127}{128}$	0.5	99.5

telophases are, at least in some cells, grossly abnormal. The division is apparently never completed, and what seems to happen is the formation of a restitution nucleus, which then degenerates. No cells in the second meiotic division, however abnormal, were ever found.

This situation is distinctly reminiscent of the male hybrids between *D. pseudoobscura* and *D. persimilis* (Dobzhansky, 1934 and 1951). Here too, "The meiotic chromosome pairing is variable; no univalents, some univalents, or only univalents may be present at the first meiotic division. Irrespective of the numbers of the bivalents and univalents formed, only a single very abnormal meiotic division takes place." Furthermore, these two cases agree in that the disturbances of the reproductive systems of the males are confined to the gonads, and the remaining parts of the reproductive system are normal. The paragonia of the sterile hybrid males contain the same binucleate cells that can be found in the paragonia of normal males. Suffice it to say, at this point, that nothing even approaching a mature spermatozoon has been seen in the F_1 males.

The males obtained in the experimental backcross generations offer an unusual cytological picture. As shown above, these males are always sterile, and their testes are so rudimentary that often individual cells within the testicular sheath cannot even be recognized. Greater abnormalities in the backcross males than in the F_1 males arise evidently because the former are the sons of hybrid females, while the latter are not. It seems that the condition of the males does not always improve with repeated backcrosses to one parental species, as it does in so many other hybrid crosses (e.g., *D. pseudoobscura* × *D. persimilis*). Yet, the small degree of sperm motility that was achieved in a few backcross males, where the testes were not rudimentary, was never found in F_1 males. Clearly, a more detailed adequate cytological analysis is called for.

Discussion

Hybrid sterility, i.e. failure to produce functional gametes, is a usual, though not invariable, property of hybrids between fully formed species. Like other reproductive isolating mechanisms, hybrid sterility admits of degrees; not infrequently only one of the sexes is sterile and the other fertile; or else, the sterility may be incomplete or may appear only in some environments but not in others. More important still, the sterility of different hybrids is produced by different causes. At least four types of hybrid sterility have been recorded, as follows (Dobzhansky, 1951):

1. Chromosomal sterility, wherein differences in the gene arrangements in the chromosomes of the forms crossed lead to mechanical difficulties in the meiotic pairing and disjunction of the chromosomes in the hybrids.
2. Genic sterility, caused by discordant genic endowments contributed by the parents of the hybrid, resulting in a physiological disturbance of the process of gametogenesis.
3. Cytoplasmic sterility, which results in failure of gametogenesis in individuals developing from eggs deposited by mothers of certain genetic constitutions.
4. Intersexuality, where the hybrids are sterile because they are neither fully formed females nor males.

The sterility of hybrids between the subspecies, or incipient species, of *Drosophila paulistorum* is of particular interest because the reproductive isolation between these forms is presumably in the process of development. These forms are, at our time level, in a state of transition between race and species (Dobzhansky and Spassky, 1959). The evidence presented above shows that the genetic basis of this sterility is a rather unusual one. All males developing from the eggs de-

posited by hybrid females are sterile, although some of these males, even in the first backcross, should carry chromosomal complements consisting of chromosomes of a single subspecies. The sterility would, accordingly, seem to be of cytoplasmic origin, superficially resembling that discovered by Laven (1953, 1959) in hybrids between geographic races of certain species of *Culex* mosquitoes. A closer scrutiny shows, however, that this resemblance is indeed superficial. In *D. paulistorum* the sterility of a male is not caused by an incompatibility of a chromosomal complement of the individual with self-reproducing cytoplasmic constituents inherited through the maternal line. It is caused, rather, by the genotype of the mother so altering the properties of the eggs she produces that the male zygotes which develop from them are sterile regardless of the chromosomal complement which they themselves come to possess after fertilization. At the same time the female zygotes developing from the same eggs grow into apparently fully fertile females. In contrast to Laven's mosquitoes, the sterility in *D. paulistorum* is, therefore, at bottom genic sterility, not cytoplasmic sterility.

One has to look elsewhere for an analogy to the sterility mechanism found in the inter-subspecific hybrids in *D. paulistorum*. Spurway (1946, 1948) reported an extreme example of a delay in gene action causing sterility in a mutant of *D. subobscura*, appropriately named "grandchildless." According to Spurway, "An autosomal recessive gene, *grandchildless*, causes no change in the phenotype of flies homozygous for it. But in the progeny of homozygous females, no matter by what father, all females have rudimentary ovaries, and are sterile, while 98.4% of the males have no testes . . . . That the mother but not the father is influential in determining whether a culture shall be sterile suggests that the character is determined by the maternal genetic constitution and not by the constitution of the abnormal flies." Spurway's description of the situation is fully applicable to *D. paulistorum*, except for the fact that in the latter the sterility affects males only and leaves the females quite normal.

The grandchildless condition in *D. subobscura* is apparently due to a single recessive gene. This is not so in *D. paulistorum*. Perhaps the most remarkable, and unexpected, aspect of the situation in the latter species is that the presence of any one of the three chromosomes, as well as all of the three chromosomes together, combined with a chromosomal complement of a foreign subspecies is sufficient to make a female deposit eggs developing into sterile males. It is as if every chromosome pair carried, in the different subspecies, complementary dominant genes, the interaction of which produced a grandchildless condition. Such a mechanism is, as far as the writer is aware, unprecedented either in species hybrids or within a single species.

Summary

Male hybrids between subspecies, or incipient species, of the *Drosophila paulistorum* complex (Centro-American, Amazonian, and Andean-South Brazilian) are completely sterile. Hybrid females are fertile and can be backcrossed to either subspecies. Experiments have been made with chromosomes of the different subspecies marked by suitable marker genes which permitted the distribution of a given chromosome to be followed through a series of repeated backcrosses. The evidence is consistent with the view that any female which carries any mixture of the chromosomes of different subspecies deposits eggs giving rise to sterile male zygotes and to fertile female ones. The sterility of a male depends, in the backcrosses, on the genetic constitution of his mother, and not on his own genetic constitution. In other words, the properties of an egg are determined, as far as the sterility of the backcross males is concerned, by the genotype present in it before meiosis, and not by that formed following fertilization. It should be noted

that the presence in the mother of just one subspecies-foreign chromosome is already sufficient to make it produce sterile sons. The situation in *D. paulistorum* is compared with that in the "grandchildless" mutant in *D. subobscura,* and with that in the hybrids between certain subspecies of *Culex* mosquitoes. It is in several respects different from either, and seems to be unprecedented in the genetic literature.

Acknowledgments

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EVOLUTION AND VARIATION IN *TRILLIUM*. VI. MIGRATIONS AMONG NATURAL POPULATIONS OF *T. KAMTSCHATICUM* ACROSS THE ISHIKARI DEPRESSION

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Three geographical population groups have been described for plants of *Trillium kamtschaticum* distributed in northern Japan (Kurabayashi, 1957). These groups meet at the Ishikari Depression where migrations occur among them (fig. 1). The migration was investigated in detail by comparative analysis of chromosome composition among populations of this plant distributed along and near the Depression. The method of analysis was the same as that described by Haga and Kurabayashi (1953). The results obtained will be reported in the following.

MIGRATIONS ACROSS THE ISHIKARI DEPRESSION

A dozen populations distributed along and near the Ishikari Depression were chosen for examination of the migrations. Six populations were investigated in the northern and southern part of the Depression: *Nk, Np, My, Sg, Ss* and *En* in the northern part; and *Ni, Sr, Hg, Nn, Nb* and *Tm* in the southern part (fig. 1).

The composition of the twelve populations in respect to chromosome types is shown in table 1. It is clearly seen from this table that populations examined in the northern part differ greatly in chromosome composition from those chosen for analysis in the southern part. Seven of the forty-four chromosome types, which were found in the six populations examined in the northern part, were included in all, four in five, four in four and one in three populations. On the other hand, only one of the forty-one types, which were detected in the six populations analyzed in the southern part, were found in all, none in five, one in four and three in three populations (table 1). It is evident from these data that the populations distributed in the northern part of the Depression reveal a greater degree of interpopulational homogeneity than those in the southern part, where interpopulational heterogeneity is conspicuous. The interpopulational homogeneity in the northern part must be maintained by genetic continuity among the populations, while the heterogeneity in the southern part is an indication of genetic discontinuity.

N. T. C. values[5] obtained between the populations of the northern and southern part of the Depression also revealed marked differences (table 2). The values of populations from the northern part varied from 2.4 to 3.4 with an average of 3.0, while those of populations from the southern part ranged from 1.0 to 2.8 with an average of 1.6.

For the purpose of representing clearly the mode of migrations across the Ishikari Depression, chromosome types were divided into nine categories according to their origin (Fukuda & Kozuka, 1958,

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[5] Homogeneity of chromosome constitution in a given natural population is expressed by number of types per chromosome: N. T. C. = total number of chromosome types found in samples of a given population/5. Here 5 is the number of homologous chromosome pairs of this plant (Hiraizumi, 1956).

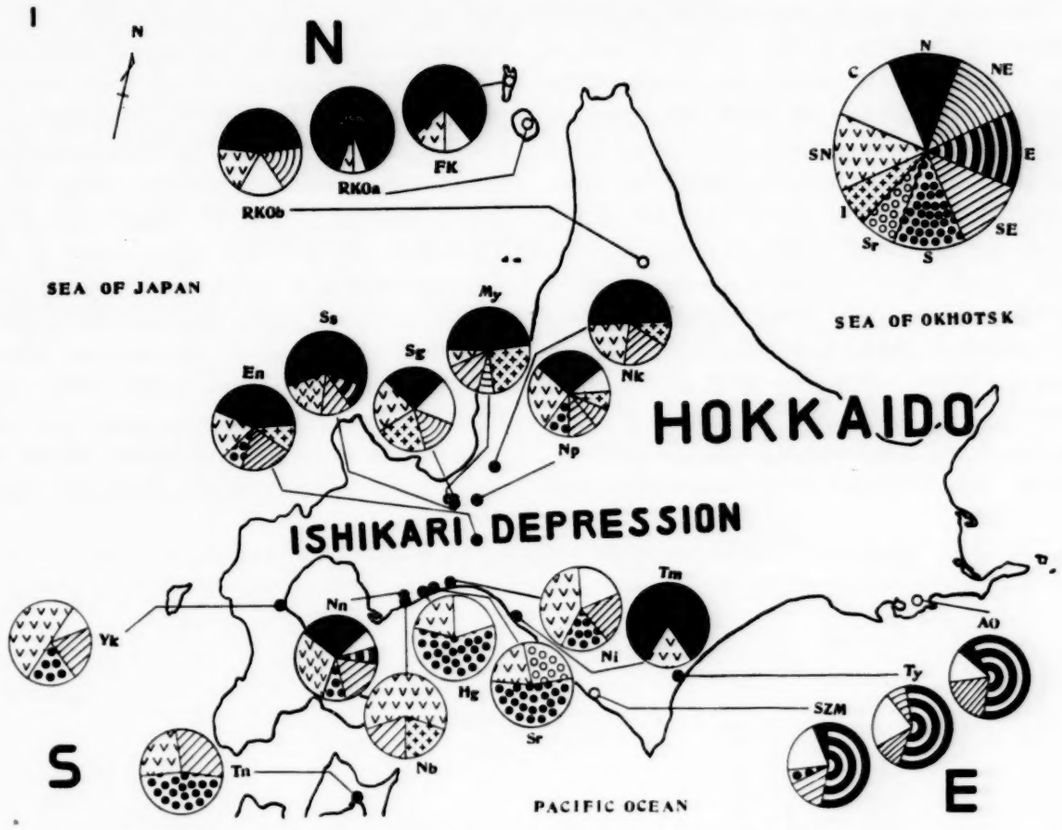

FIG. 1. Fan diagram representing chromosome composition of populations and subgroups of populations belonging to **North**, **South** and **East** population groups together with that of populations distributed in the Ishikari Depression. For convenience in drawing the figure, chromosome types whose frequencies do not attain more than five per cent of the total found in a given population or subgroup of populations were omitted from the diagram. A black dot represents a population and an empty one a subgroup of populations. The fan diagram at the upper right in the map represents nine categories of chromosome types distinguished according to their mode of distribution. **N, E** and **S**: chromosome types which are restricted to **North, South** and **East**. **NE, SE,** and **SN**: those commonly found in **North** and **East, South** and **East** and **South** and **North.** **C**: those found commonly in the three population groups. **I**: the types found restrictedly among populations in the Ishikari Depression. **Sr**: those found in *Sr* and neighboring populations.

and table 3 in this paper). One can then construct a fan diagram representing the chromosome composition of each of the populations examined in the Ishikari Depression (fig. 1). The chromosome composition of populations and subgroups of populations in **North, South** and **East** population groups (Kurabayashi, 1957) are also shown for comparison.

Population *Ty* and subgroups *AO* and *SZM* which belong to **East** are characterized by types endemic to this population group: the frequency of the endemic types (**E**) attaining more than half of the total ones detected in each. More than three quarters of the chromosome types examined in *FK* and *RKO*a and about half of those in *RKO*b are characterized by **N** types which are endemic to **North.** The populations belonging to **South,** namely, *Tn* and *Yk* reveal the highest interpopulational discontinuity among those of the three populational groups. The genetic continuity, however, is indicated by the maintenance of a few endemic types, **S**'s (Kurabayashi, 1957, and Fukuda and Kozuka, 1958). The population groups, **East, North** and **South,** meet at the Ishikari Depression across which migrations have occurred.

The chromosome composition of populations distributed in the northern part of the Depression is influenced mostly from **North.** Frequency of types belonging to **N, NE** and **SN** account for more than half of all the types included in these populations, and the types of **N** are found in all the six populations in high frequencies (more than a quarter of the total in each). Migration from **South** is less conspicuous than that from the **North** and the types belonging to **S** attain more than five per cent only in two of the six populations. **East** contributes the least influence and **E** types attain more than five per cent only in population *Ss*. (This grows natively in the Botanical Garden of Hokkaido University. However, a transplant station of *Trillium* has existed since 1942 in this Garden. The first transplantation of *T. kamtschaticum* was made from *Sz* (belonging to **East**) in 1942. Therefore, it is likely that some of the **E** types found in this population may be introduced artificially, because the examination of chromosome composition of *Ss* was carried out in the year 1954, viz., after 11 flowering seasons since the first transplantation.) Several types which are endemic to the Depression (**I**) are found

TABLE 1. *Chromosome composition of natural populations distributed in the Ishikari Depression*

Chromosome	Type	Geographical group	Populations											
			Northern part						Southern part					
			Nk	*Np*	*My*	*Sg*	*Ss*	*En*	*Ni*	*Sr*	*Hg*	*Nn*	*Nb*	*Tm*
A	*1*	**N**	2	6	—	5	30	10	—	—	—	—	—	18
	52	**N**	14	—	2	—	—	—	—	—	—	—	—	—
	51	**N**	1	—	3	—	—	—	—	—	—	—	—	—
	18	**N**	1	—	—	—	—	—	—	—	—	—	—	—
	54	**N**	—	2	—	—	—	—	—	—	—	1	—	—
	38	**SN**	8	18	—	6	—	1	30	—	—	58	16	—
	13	**S**	—	—	—	—	—	—	—	44	11	1	—	—
	33	**E**	—	—	—	—	1	—	—	—	—	—	—	—
	29	**E**	—	—	—	—	2	—	—	—	—	—	—	—
	40	**E**	—	—	—	—	—	1	—	—	—	—	—	—
	36	**E**	—	—	—	—	—	—	—	—	—	1	—	—
	39	**E**	—	—	—	—	—	—	—	—	—	2	—	—
	53	**I**	14	14	15	29	2	12	—	—	—	—	—	—
	50	**I**	2	—	—	8	—	—	—	—	—	—	—	—
	14	**Sr**	—	—	—	—	—	—	—	56	—	—	—	—
	11	**C**	1	—	—	1	1	—	—	—	—	—	—	—
	69	**C**	1	—	—	—	—	—	—	—	—	—	—	—
	25	**C**	—	—	—	1	—	—	—	—	—	—	—	—
	24	**C**	—	—	—	—	—	—	—	—	—	29	—	—
B	*9*	**N**	38	1	2	1	2	1	—	10	—	9	—	—
	13	**N**	—	20	18	12	26	14	—	—	—	1	—	—
	21	**N**	2	—	—	—	—	—	—	—	—	—	—	—
	16	**N**	—	—	—	—	—	—	—	—	—	—	—	18
	1	**SN**	—	—	—	—	—	4	—	—	—	73	16	—
	7	**S**	—	—	—	—	—	—	—	18	—	—	—	—
	12	**SE**	—	—	—	—	—	—	—	—	—	2	—	—
	17	**E**	—	—	—	—	1	—	—	—	—	—	—	—
	6	**Sr**	2	—	—	—	—	—	—	60	—	3	—	—
	11	**Sr**	—	—	—	—	—	—	—	1	—	—	—	—
	8	**Sr**	—	—	—	—	—	—	—	7	—	—	—	—
	10	**C**	1	3	—	35	3	1	—	4	—	1	—	—
	2	**C**	—	13	—	1	3	3	30	—	11	3	—	—
	3	**C**	—	3	—	1	1	1	—	—	—	—	—	—
	20	**C**	1	—	—	—	—	—	—	—	—	—	—	—

TABLE 1. *Chromosome composition of natural populations distributed in the Ishikari Depression—(Continued)*

Chromosome	Type	Geographical group	Populations											
			Northern part						Southern part					
			Nk	*Np*	*My*	*Sg*	*Ss*	*En*	*Ni*	*Sr*	*Hg*	*Nn*	*Nb*	*Tm*
C	*11*	**N**	1	6	14	5	8	8	—	—	—	2	—	—
	10	**N**	1	1	—	—	—	—	—	—	—	80	1	—
	17	**N**	2	—	—	—	—	—	—	—	—	—	—	—
	6	**SN**	40	33	6	45	26	15	30	100	11	8	15	18
	9	**SE**	—	—	—	—	—	—	—	—	—	1	—	—
	13	**E**	—	—	—	—	2	—	—	—	—	—	—	—
	1	**E**	—	—	—	—	—	1	—	—	—	—	—	—
	2	**E**	—	—	—	—	—	—	—	—	—	1	—	—
	4	**E**	—	—	—	—	2	—	—	—	—	—	—	—
D	*1*	**N**	43	19	8	35	21	15	—	—	—	30	—	18
	3	**S**	—	21	2	—	5	9	30	27	11	39	—	—
	7	**S**	—	—	—	—	—	—	—	73	—	2	—	—
	4	**E**	1	—	—	—	4	—	—	—	—	17	—	—
	14	**E**	—	—	—	—	—	—	—	—	—	1	—	—
	11	**I**	—	—	—	—	6	—	—	—	—	—	3	—
	15	**I**	—	—	9	11	—	—	—	—	—	—	1	—
	16	**I**	—	—	1	2	—	—	—	—	—	—	12	—
	9	**C**	—	—	—	2	—	—	—	—	—	3	—	—
E	*19*	**N**	1	—	—	—	—	—	—	—	—	—	—	—
	17	**N**	—	—	—	—	9	—	—	—	—	—	—	8
	9	**N**	—	—	—	—	—	—	—	—	—	—	—	10
	2	**S**	—	—	—	—	—	—	—	100	11	—	—	—
	1	**SE**	37	14	14	7	6	11	—	—	—	42	16	—
	3	**SE**	1	11	—	3	12	12	30	—	—	43	—	—
	11	**E**	2	—	—	—	—	—	—	—	—	2	—	—
	10	**NE**	1	15	6	40	7	1	—	—	—	1	—	—
	6	**C**	2	—	—	—	2	—	—	—	—	4	—	—
Total chromosome sets			44	40	20	50	36	24	30	100	11	92	16	18

in five out of the six populations in fairly high frequencies. Each of the populations in the northern part includes types belonging to more than four categories, each of which contained types with a total frequency of more than five per cent. The proportion of types belonging to the different categories differs from population to population. The difference, however, is far less in degree than is found in populations examined in the southern part.

Migration from **South** is clearly evident in four out of the six populations examined in the southern part. The *Tm* population is overwhelmingly comprised of elements of **North.** More than a quarter of the chromosome types found in *Sr* are the ones inherent to the population. The types endemic to the Depression are included only in the population *Nb*. The *Nn* population has the elements of **N** and **E.** The latter is restricted, in the southern part, to this population. The categories represented and their freqencies differ greatly among the populations, as just described, indicating a marked interpopulational heterogeneity.

DISCUSSION

Migrations across the Ishikari Depression have occurred in different manners between the northern and southern parts, as described above. Considering the geo-

TABLE 2. *N. T. C. values in natural populations of* T. kamtschaticum

Distribution area	Population	N. T. C.	Population group	Population & subgroup	N. T. C.
Northern part of the Ishikari Depression	*Nk*	3.0			
	Np	3.0		*FK*	1.2–2.2
	My	2.8	**North**	*RKOa*	1.2–1.7
	Sg	3.1		*RKOb*	1.2–2.3
	Ss	3.4			
	En	2.4	**South**	*Yk*	1.7
				Tn	2.5
Southern part of the Ishikari Depression	*Ni*	1.0			
	Sr	2.0		*AO*	3.9
	Hg	1.0	**East**	*Ty*	3.0
	Nn	2.8*		*SZM*	4.2
	Nb	1.7			
	Tm	1.2**			

* The N. T. C. value of *Nn* was erroneously given as 2.2 in the previous paper (Kurabayashi, 1958, p. 298, table 5), and was corrected here.

** The N. T. C. value of *Tm* was revised from the previous one on the basis of additional data: the number of plants examined in this population being increased from 4 (Kurabayashi, 1957, pp. 40–45) to 9 (Fukuda and Kozuka, 1958, p. 295, Appendix, table 16).

graphical position (fig. 1), it is not surprising that the populations in the northern part of the Depression are influenced mostly from **North,** while those in the southern part include elements originating from **South.** Contributions from **East** are smaller than those from **North** and **South.** It seems that the Hidaka mountain range, which terminates at the cliff of Mt. Apoi (fig. 2), may have acted as an effective barrier preventing migrations from **East.** The Depression was repeatedly submerged during Interglacial Ages. The most recent connection between the northern and the southern parts has been established in the last few thousand years. The accumulation of river sediments under conditions of a somewhat lower sea level has been the main factor responsible for the connection of the northern part. Thus habitats suitable for this plant have developed there allowing continuous migrations from **North.** On the other hand, the connection in the southern part has been promoted by products of volcanoes on the south-western side of the Depression (fig. 2). The accumulation of fresh products of volcanoes must have destroyed most suitable habitats. Thus the migrations in the southern part might have been fragmental ones interrupted by volcanic activities. Repeated showering by volcanic ashes must have kept the effective size of populations of this plant small. It might also be that populations would frequently have been eliminated by eruptions of volcanoes and then re-established between eruptions from a meager seed source that may have survived (cf. Stebbins, 1950, pp. 147-148).

Continuous migrations in the northern part naturally have resulted in interpopulational homogeneity among populations distributed there, while fragmental ones in the southern part have brought about interpopulational heterogeneity. The heterogeneity may also be conspicuous in the case of chance survival passing through a bottle neck provided by a volcanic eruption. The difference in conditions of migration and habitation (see above) is also responsible for the difference in N. T. C. values, larger values being found among populations in the northern part, while smaller ones are found among those in the southern part (table 2).

The distribution of types endemic to the Depression (**I**) seems also to be in

accord with the ecological conditions described above. These types are included in all the populations examined in the northern part (table 1), while they are restricted to one population (*Nb*) in the southern part. It may be possible that these types recently became established as a result of mutation and selection. The populations in the northern part are so large that the observed types may be the result of mutation and selection, while random genetic drift may have eliminated

TABLE 3. *Grouping of chromosome types according to their origin* *

Chromosome	Type and category							
A	*1*	**N**	*15*	**E**	*29*	**E**	*43*	**E**
	2	**E**	*16*	**E**	*30*	**E**	*44*	**E**
	3	**E**	*17*	**E**	*31*	**E**	*45*	**E**
	4	**E**	*18*	**N**	*32*	**E**	*46*	**E**
	5	**E**	*19*	**E**	*33*	**E**	*47*	**E**
	6	**E**	*20*	**E**	*34*	**E**	*48*	**E**
	7	**E**	*21*	**SE**	*35*	**E**	*49*	**E**
	8	**E**	*22*	**S**	*36*	**E**	*50*	**I**
	9	**E**	*23*	**E**	*37*	**E**	*51*	**N**
	10	**E**	*24*	**C**	*38*	**SN**	*52*	**N**
	11	**C**	*25*	**C**	*39*	**E**	*53*	**I**
	12	**E**	*26*	**E**	*40*	**E**	*54*	**N**
	13	**S**	*27*	**E**	*41*	**E**	*55*	**E**
	14	**Sr**	*28*	**E**	*42*	**E**	*69*	**C**
B	*1*	**SN**	*7*	**S**	*12*	**SE**	*17*	**E**
	2	**C**	*8*	**Sr**	*13*	**N**	*18*	**E**
	3	**C**	*9*	**N**	*14*	**E**	*19*	**S**
	4, 5	**E**	*10*	**C**	*15*	**N**	*20*	**C**
	6	**Sr**	*11*	**Sr**	*16*	**N**	*21*	**N**
C	*1*	**E**	*5*	**E**	*9*	**SE**	*13*	**E**
	2	**E**	*6*	**SN**	*10*	**N**	*14*	**E**
	3	**E**	*7*	**E**	*11*	**N**	*15*	**E**
	4	**E**	*8*	**E**	*12*	**N**	*17*	**N**
D	*1*	**N**	*5*	**E**	*9*	**C**	*13*	**E**
	2	**E**	*6*	**E**	*10*	**E**	*14*	**E**
	3	**S**	*7*	**S**	*11*	**I**	*15*	**I**
	4	**E**	*8*	**E**	*12*	**E**	*16*	**I**
E	*1*	**SE**	*6*	**C**	*11*	**E**	*16*	**E**
	2	**S**	*7*	**S**	*12*	**E**	*17*	**N**
	3	**SE**	*8*	**E**	*13*	**E**	*19*	**N**
	4	**E**	*9*	**N**	*14*	**E**		
	5	**C**	*10*	**NE**	*15*	**E**		

* The method of grouping of types into each of the categories is the same as that adopted in the previous paper (Fukuda and Kozuka, 1958). Some additions and revisions, however, were made in grouping the types with reference to the results of the population analysis carried out in 1958.

Additions: A-*69*, B-*19*, B-*20*, B-*21*, C-*17*, E-*19*

Revisions:

Chromosome type	Category	
	Prerevised	Revised
A-*51*	**I**	**N**
A-*52*	**I**	**N**
B-*9*	**I**	**N**
E-*6*	**E**	**C**

E-*10* was corrected from **SE** to **NE** because the former was a misprint.

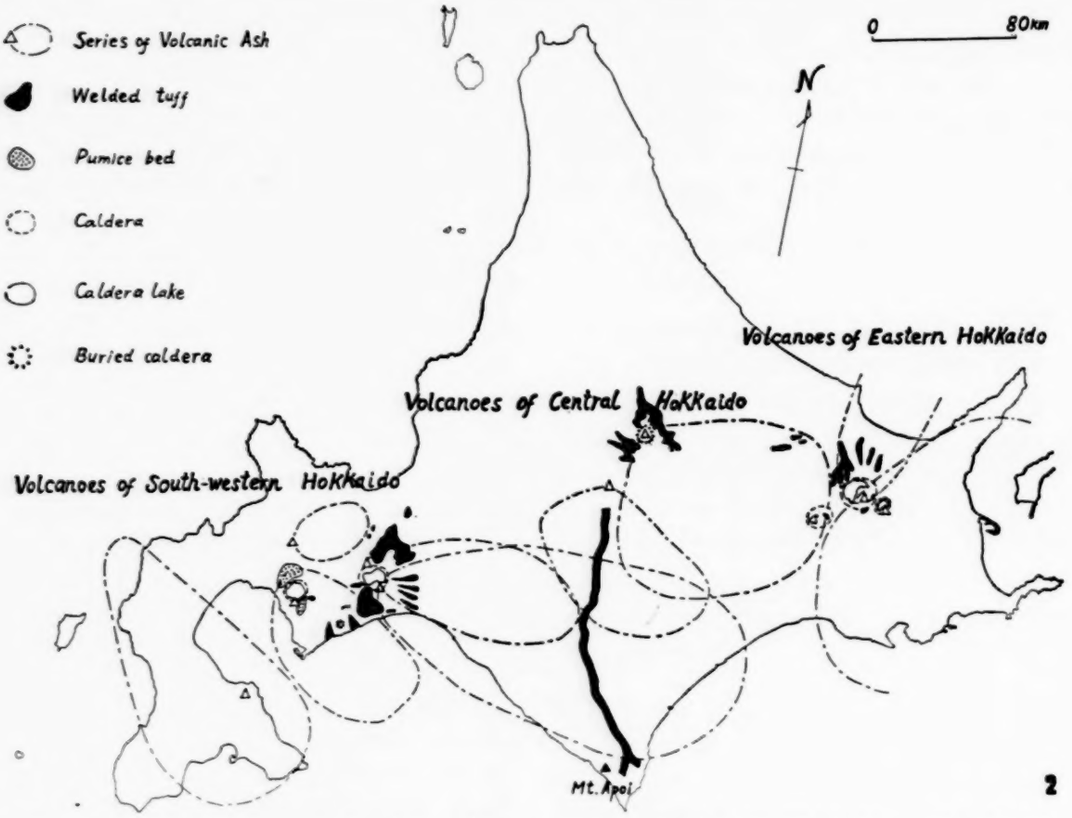

FIG. 2. A simplified map showing volcanoes and mountains in Hokkaido. The Hidaka mountain range is shown by a thick line drawn from north to south across the southern half of central Hokkaido.

the majority of mutant types from the populations in the southern part where limitation in population size is conspicuous. It is also possible, however, that some of the types belonging to this category are migrants from other districts, because not all the populations of this plant have yet been examined.

In conclusion, the conditions realized among natural populations of *T. kamtschaticum* examined in the northern and southern part of the Ishikari Depression indicate various modes of interactions between natural selection and random genetic drift (used in a broad sense; cf. Dobzhansky and Pavlovsky, 1957) in the course of migrations. Continuous migrations taking place in a continuous habitat may lead to an equilibrium. Each of the migrant chromosome types may be retained in a given population at a balance between migration pressure and selective advantage. When migrations are interrupted and habitats become discontinuous, the balance is broken and random genetic drift starts. Chance survival of colonies in isolated areas may promote discontinuity, because these colonies are apt to be different from each other from the time that they are re-established. The marked interpopulational heterogeneity among populations examined in the southern part of the Depression may, therefore, represent the result of chance survival under conditions of a limited supply of genetic variability. If chromosome types from different regions are present in small populations in various frequencies, one or a few of the types may survive and be fixed in each of the populations due to random genetic drift which will begin to operate at once with cessation of migration. Selection may then act in such a limited manner as to result in fixed adaptation (Kurabayashi, 1958) to a certain adaptative peak available to

each population. The habitats of *T. kamtschaticum* are more heterogeneous in the southern part than in the northern part in respect to edaphic conditions, because of volcanic activity in the former. This divergence might have promoted the interpopulational heterogeneity among the populations in the southern part leading to fixed adaptation in each population.

Summary

Three geographical population groups, **North, South** and **East,** of *Trillium kamtschaticum* distributed in northern Japan meet at the Ishikari Depression where migrations have occurred among them. The migrations were examined by comparative analysis of chromosome composition in natural populations of this plant found there.

In the northern part of this Depression, where river sediments have afforded habitats suitable for this plant, continuous migrations from **North** were conspicuous. The populations examined there were large in size and revealed interpopulational homogeneity.

Each of the populations distributed in the southern part of the Depression was small in size and high in intrapopulational homogeneity. Their chromosome composition, however, differed greatly from one another. Such situation may be the result of random genetic drift preceded by chance survival of populations in areas subjected to volcanic activities, which have deprived this plant of its habitats. Migrations in this region have been fragmental because they were disturbed by eruptions of volcanoes situated on the south-western side of the Depression.

The results of the comparative examinations carried out in the present study elucidated various modes of interactions between selection and random genetic drift in the course of migrations. The influence of the former was apparent in the northern part, that of the latter was markedly evident in the southern part of the Ishikari Depression.

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EVOLUTION AND VARIATION IN *TRILLIUM*. VII. MIGRATIONS BETWEEN NORTHERN AND EASTERN POPULATION GROUPS OF *T. KAMTSCHATICUM*

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In the foregoing paper of this series (Fukuda *et al.*, 1960), migrations among natural populations of *Trillium kamtschaticum* across the Ishikari Depression, a narrow depressed area connecting the southern part of Hokkaido with the central part, were investigated. The present paper deals with migrations that have taken place in the north-eastern region (Kitami Province) of Hokkaido, where the northern population group (**North**) of this plant borders on the eastern one (**East**). The front of the contact of these two groups ranges from the seashore of Okhotsk, passing through the interior of Kitami Province, to the central part of Hokkaido, where volcanoes of central Hokkaido divide the two groups (fig. 2 in Fukuda *et al.*, 1960 and fig. 1 in the present paper). Seven populations, *Ke, Op, Sk, Ab-1, Ab-2, Mb* and *Tp*, distributed in the coastland facing the sea of Okhotsk and two, *Bk* and *Ot*, from the coastland of Nemuro Province, were chosen for examination. Also, four populations *Eg, Ok, An* and *Kt*, were taken from the interior of Kitami Province (fig. 1). The migrations were examined by making analyses of chromosome composition of these populations.

EXAMINATION OF THE MODE OF MIGRATIONS

Chromosome composition of the populations examined is shown in table 1. Here the chromosome types of each pair are grouped into nine categories according to the origin of each type (Fukuda and Kozuka, 1958). The majority of the types fall into six categories: **SN, N, NE, SE** and **C. SN** and **N** are the categories established in **North** (northern population group) and **E** and **SE** are those in **East** (eastern population group). The frequencies of the former among populations distributed in the coastland of Okhotsk go down, in chromosome A, C and D, from north-west to south-east along the coastline, while those of the latter change in the reverse direction. No such clinal changes are seen in chromosomes B and E. It is supposed that the presence of types belonging to the category **C** in B and that of **NE** in E may interfere with the manifestation of clinal changes (fig. 2). The populations taken from the interior of Kitami Province are also composed mostly of chromosome types belonging to the six categories mentioned above. The populations from the coastland of Nemuro Province are derived more from **East** than the others.

Chromosome types belonging to categories other than the above six were found in 10 out of the 13 populations examined. The fact that types belonging to **I, S** and **Sr** are found there throws some doubt upon the method of grouping of chromosome types adopted here and elsewhere (Fukuda and Kozuka, 1958). The frequencies of these types, however, are so small that re-examination of the grouping method should be postponed until we can obtain sufficient data for the re-examination. A total of 51 types of chromosome A were omitted from the grouping in population *Tp* and *Bk*. They are, at present, found only in these popu-

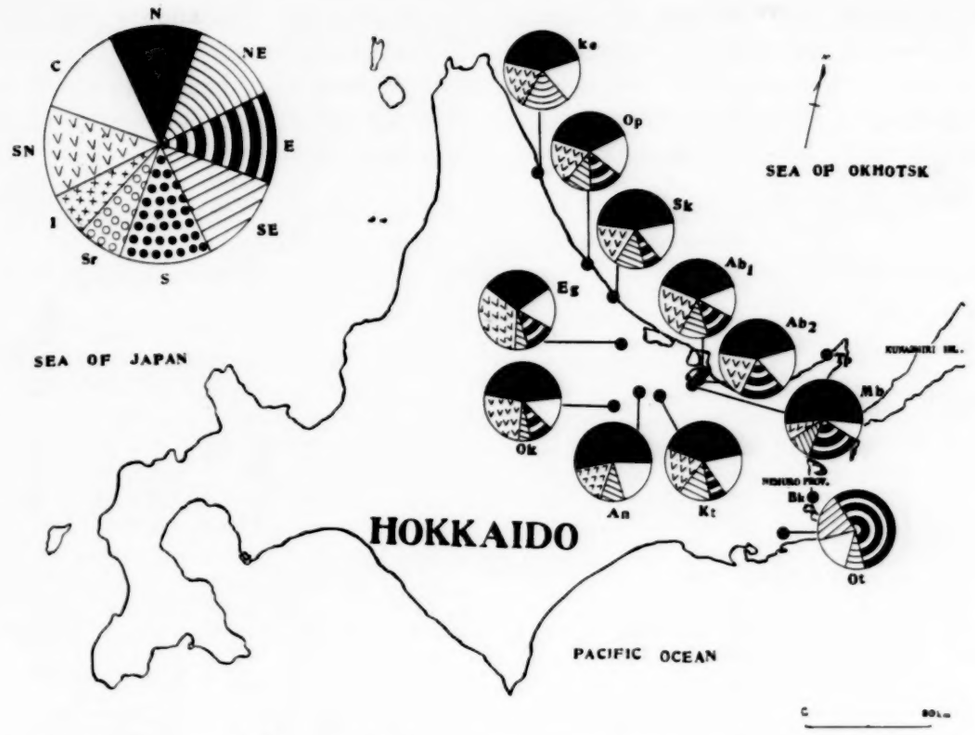

FIG. 1. A map of Hokkaido showing localities (black dots) where natural populations of *T. kamtschaticum* chosen for the present investigation were taken. Chromosome composition of the populations are represented with fan diagrams in which different types of each chromosome pair are grouped into nine categories indicated in the upper left of this figure. When a total frequency of types belonging to a category in each population does not attain 5 per cent of the total types, the category is omitted from the drawing.

lations. *34* out of the 51 types carry an arm of type *311* (fig. 1 in Fukuda and Kozuka, 1958). We are not yet sure how widely this arm type is distributed, because there are many populations of *T. kamtchaticum* left unexamined in the area from which these two populations were taken. However, it seems likely that this arm may not belong to a category other than **E**, because it has three euchromatic dots in its differential segment, and no three dotted arm types were found among natural populations in **North** and **South** (Kurabayashi, 1957).

For the purpose of representing the mode of migrations clearly we have shown the relative frequency (per cent) of (**SN** + **N**), **NE** and (**E** + **SE**) in each population with rod diagrams (fig. 2). Mixed composition and the clinal changes in frequencies of types belonging to the three groups may evidently be seen. Another attempt was made to show chromosome composition in each population with fan diagrams (fig. 1).

DISCUSSION

The evidence of migrations between **North** and **East** is clearly seen in table 1 and figures 1 and 2. The migrations are continuous ones, especially along the coast of Okhotsk where northern and eastern elements alternate with each other, forming a geocline. It is interesting to note (see bottom of fig. 2) that the N. T. C. values (cf. Hiraizumi, 1956) of the coastland populations also reveal a clinal change increasing from north-west to south-east along the coastline of Okhotsk. The increase in N. T. C. values is supposed to have been brought about by increase in population size from north to east. This is convincing if we examine the difference in vegetation be-

tween northern and eastern Hokkaido. The regression of conifers, which are predominant in northern Hokkaido, towards eastern Hokkaido where deciduous woods cover hills and lowlands, provides abundant and large habitats for *T. kamtschaticum*. This plant prefers half shade in thin deciduous woods rather than the dense shade of conifer or mixed deciduous woods.

TABLE 1. *Chromosome composition of natural populations of* T. kamtschaticum *distributed in north-eastern regions of Hokkaido.*

			Populations												
			Inland of Kitami				Coastland of Okhotsk							Coastland of Nemuro	
Chromosome	Type	Geographical group	*Eg*	*Ok*	*An*	*Kt*	*Ke*	*Op*	*Sk*	*Ab*$_1$	*Ab*$_2$	*Mb*	*Tp*	*Bk*	*Ot*
A	*38*	**SN**	22	26	37	—	—	9	17	25	26	4	4	3	2
	1	**N**	6	10	13	5	14	19	14	10	6	20	1	8	1
	18	**N**	—	—	—	—	—	—	5	7	—	12	1	2	6
	52	**N**	—	—	—	—	—	—	4	—	—	10	1	—	—
	51	**N**	—	2	—	—	—	—	—	—	—	1	—	—	—
	3	**E**	—	—	—	4	—	—	—	—	—	2	4	2	1
	31	**E**	—	—	—	—	—	—	—	—	—	1	2	—	—
	44	**E**	—	—	—	—	—	—	—	—	—	1	—	—	—
	28	**E**	—	—	—	—	—	—	—	—	—	1	—	—	11
	4	**E**	—	1	—	—	—	—	—	—	—	—	—	—	—
	2	**E**	—	—	—	—	—	—	—	—	—	—	4	—	—
	39	**E**	—	—	—	—	—	—	—	—	—	—	—	1	—
		Other **E**'s*	—	—	—	—	—	—	—	—	—	—	1	—	72
	57–73†		—	—	—	—	—	—	—	—	—	—	43	8	—
	24	**C**	—	—	—	—	—	—	—	—	—	—	—	2	6
	25	**C**	—	1	—	—	—	—	—	—	—	—	—	—	1
	53	**I**‡	—	—	—	3	—	—	—	—	—	—	1	—	—
B	*1*	**SN**	1	3	—	—	—	—	6	—	—	—	2	—	6
	13	**N**	—	—	—	3	1	1	—	4	—	17	—	2	—
	9	**N**	—	9	—	—	—	—	—	—	3	13	1	—	—
	21	**N**	—	—	—	—	—	—	—	—	—	5	—	—	—
	14	**E**	2	—	—	—	—	—	—	—	—	—	—	—	—
	17	**E**	—	—	—	—	—	—	—	5	—	—	—	—	2
	18	**E**	—	1	—	—	—	—	—	—	—	—	—	—	—
	12	**SE**	—	—	—	—	—	—	—	3	—	—	—	5	29
	2	**C**	16	22	35	7	11	26	28	21	—	2	17	6	35
	3	**C**	9	1	15	2	2	—	—	5	21	—	40	13	27
	20	**C**	—	—	—	—	—	1	4	2	2	—	2	—	—
	10	**C**	—	3	—	—	—	—	—	—	5	14	—	—	—
	7	**S**‡	—	—	—	—	—	—	2	2	1	1	—	—	1
	6	**Sr**‡	—	1	—	—	—	—	—	—	—	—	—	—	—

* A total frequency of types belonging to **E** found in population *Ot* and not in the others listed in this table (cf. Fukuda and Kozuka, 1958, Appendix table 34).

† These types were not grouped in any of the nine categories because the types were found only in populations *Tp* and *Bk*, both of which are taken from the district where many populations of *T. kamtschaticum* are left unexamined.

‡ These types have not been revised from the original categories, because their frequencies were always small in the populations examined here.

§ These types were omitted from grouping because each of them was found only once in a single population.

TABLE 1. *Chromosome composition of natural populations of* T. kamtschaticum *distributed in north-eastern regions of Hokkaido.—(Continued)*

Chromosome	Type	Geographical group	Populations												
			Inland of Kitami				Coastland of Okhotsk							Coastland of Nemuro	
			Eg	*Ok*	*An*	*Kt*	*Ke*	*Op*	*Sk*	*Ab*$_1$	*Ab*$_2$	*Mb*	*Tp*	*Bk*	*Ot*
C	*6*	**SN**	25	23	3	2	14	15	14	24	6	14	3	3	—
	17	**N**	2	—	—	—	—	5	19	17	—	—	—	—	—
	10	**N**	1	13	47	2	—	8	7	1	19	—	1	—	—
	11	**N**	—	2	—	1	—	—	—	—	—	—	—	—	—
	12	**N**	—	1	—	1	—	—	—	—	—	—	—	—	—
	13	**E**	—	—	—	—	—	—	—	—	5	30	27	1	20
	3	**E**	—	—	—	—	—	—	—	—	2	7	13	22	75
	1	**E**	—	1	—	—	—	—	—	—	—	—	9	—	5
	2, 4, 5	**E**	—	—	—	—	—	—	—	—	—	—	7	—	—
	9	**SE**	—	—	—	6	—	—	—	—	—	—	2	—	—
	16§		—	—	—	—	—	—	—	—	—	1	—	—	—
D	*1*	**N**	28	35	50	12	14	28	40	42	29	44	23	25	9
	2	**E**	—	2	—	—	—	—	—	—	—	—	2	—	—
	4	**E**	—	—	—	—	—	—	—	—	3	3	13	—	1
	5	**E**	—	—	—	—	—	—	—	—	—	2	16	—	90
	8	**E**	—	—	—	—	—	—	—	—	—	—	7	—	—
	9	**SE**	—	—	—	—	—	—	—	—	—	—	1	—	—
	3	**S**‡	—	3	—	—	—	—	—	—	—	3	—	1	—
E	*19*	**N**	—	19	—	—	—	—	—	—	12	18	—	—	—
	9	**N**	—	—	22	—	—	—	—	—	—	—	—	—	—
	10	**NE**	4	1	8	2	14	—	—	—	—	—	49	9	34
	14	**E**	—	2	—	—	—	—	—	—	—	—	—	—	—
	16	**E**	—	—	—	—	—	—	—	—	1	—	—	—	—
	11	**E**	14	9	2	—	—	20	14	20	13	8	—	—	—
	1	**SE**	1	7	14	10	—	8	26	22	6	20	1	—	30
	3	**SE**	7	1	4	—	—	—	—	—	—	6	11	17	36
	6	**C**	1	1	—	—	—	—	—	—	—	—	—	—	—
	2	**S**‡	1	—	—	—	—	—	—	—	—	—	—	—	—
	18§		—	—	—	—	—	—	—	—	—	—	1	—	—
Total chromosome sets			28	40	50	12	14	28	40	42	32	52	62	26	100

As discussed by the junior author elsewhere (Kurabayashi, 1957, 1958), populations in **North** have small N. T. C. values due to the interaction between random genetic drift and natural selection. The effect of the former may be decreased with an increase in population size, while the latter may become more effective. Thus different equilibria between selection and migration may be attained in each chromosome pair of different populations.

The migrations among populations on the coast of Kitami Province may have taken place *via* the coastline along the slope of marine terraces. The pathway through the hinterlands may have been restricted by the dark conifer woods. We have then an example of directional migrations among populations in a row.

Manifestation of a cline ought to be most apparent in such linear migrations. On the other hand populations in the interior must have been exposed to migrations of various directions, and for this reason may have failed to form a geocline.

The above mentioned clinal changes among the populations distributed along the coast of Okhotsk stands in sharp contrast to the abrupt change in the southern part of the Ishikari Depression (Fukuda *et al.*, 1960). This discontinuity was ascribed to a marked influence of random genetic drift, which has been promoted by volcanic activities in the southern part. There are active volcanoes also in eastern Hokkaido (cf. fig. 2 in Fukuda *et al.*, 1960). Their products cover wide areas where some of the populations chosen for the present investigation were taken. We failed, however, to find any influence of the volcanic activities on reduction of population size. It may be that the depth of the cover of volcanic products was not sufficient to destroy this plant or that the entire area was not covered in any one eruption thereby permitting survival of populations of sufficient size to escape the

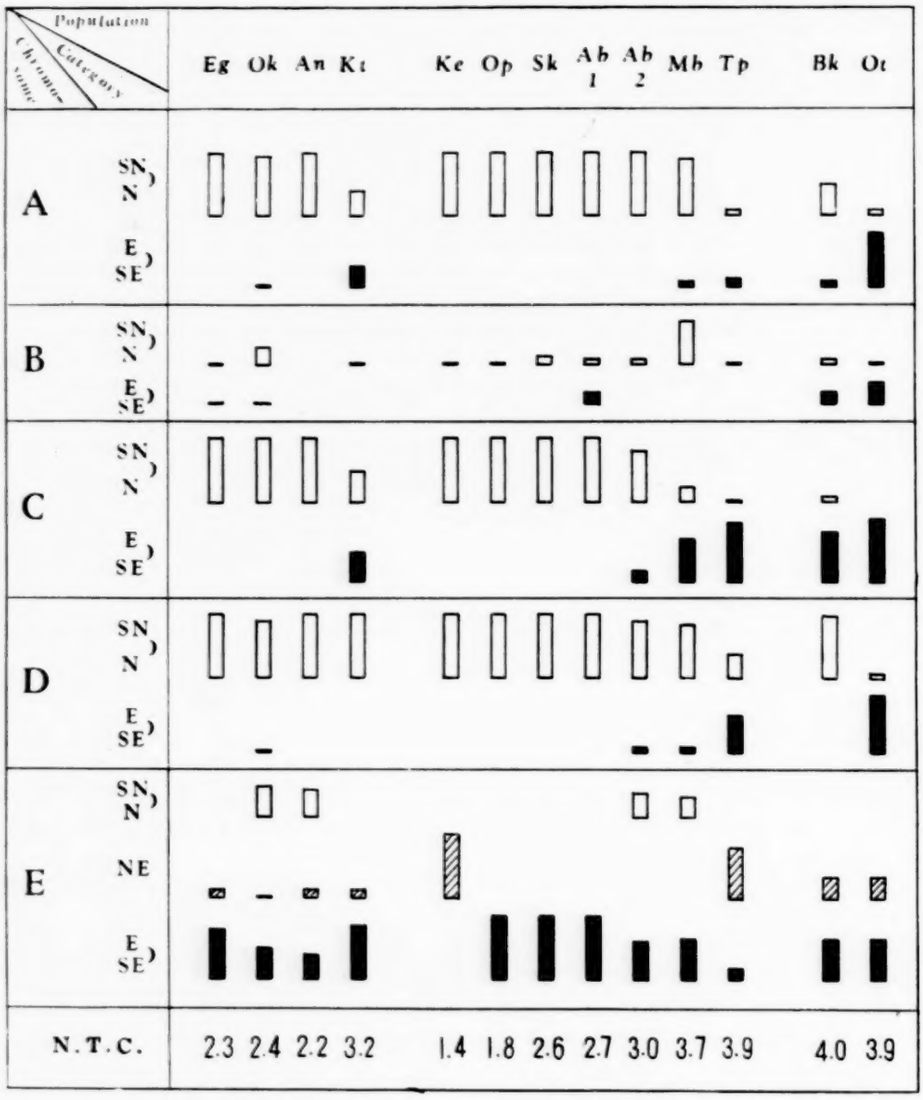

FIG. 2. Frequency (per cent) of types of **(SN + N)**, **NE** and **(E + SE)** of each chromosome pair in the 13 populations. Types of categories other than the above five are omitted from this table.

effect of random genetic drift. Eruptions of volcanoes of central and eastern Hokkaido have been none the less large in scale as compared with those of southern Hokkaido, where the eruptions have suffered marked influence upon the migrations across Ishikari Depression (Fukuda *et al.*, 1960). In the latter case, however, the route of migration must have been blocked effectively at the bottle neck at the southern side of the depression.

Acknowledgment

We are deeply indebted to Professor Harlan Lewis, University of California, Los Angeles, for valuable criticism and advice on the manuscript. Thanks are also due to Professor Misao Tatewaki, Director of the Botanical Garden, Hokkaido University, Sapporo, who has provided facilities for a transplant station of *Trillium* in the Botanical Garden.

Summary

Migrations between northern and eastern population group of *Trillium kamtschaticum* were examined by making comparative analysis of chromosome composition among populations of this plant distributed in north-eastern regions of Hokkaido.

A fine geocline was demonstrated along the coastline of sea of Okhotsk. The cline is supposed to be maintained by a balance between selection and migration pressure in each of the populations, arranged in a row, from north-west to south-east along the coastline.

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THE BREEDING GROUP AND SEED STORAGE: A STUDY IN POPULATION DYNAMICS

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This is a report on the yearly frequencies since 1941 of blue and white flowered plants of *Linanthus parryae*, a conspicuous annual of the Mojave desert. Like most desert annuals, its abundance and dispersion varies greatly from year to year and from place to place in response to weather. The size of the breeding group would be expected to vary with these expansions and contractions. An initial survey (Epling and Dobzhansky, 1942) suggested that the inheritance of color depends on one pair of alleles. Having these natural markers, the species appeared to be a useful subject with which to study the effects of selection and genetic drift.

The initial survey sampled a naturally delimited area of about 600 square miles. *Linanthus* was extraordinarily abundant in 1941 and the population was almost literally continuous throughout the area (fig. 1). For the most part it consisted of white flowered plants. Three mixed areas were found, however, in which the color frequencies were locally highly variable. Only occasional blue individuals or small patches were observed in some parts of the white area. Subsequent sampling has shown that these mixed areas are persistent and that their limits have not materially changed. Four years sampling of the westernmost mixed area by the coarse method used in the initial survey also indicated that color frequencies tended to remain constant in very local populations. Permanently marked experimental plots were accordingly constructed within this area, which have permitted a systematic and detailed sampling of dispersion and flower color each year since 1944.

The exact mode of inheritance of flower color is still uncertain. With very few exceptions, blue and white are readily distinguished in the field and under cultivation, not only in living plants but also by the dried corollas. Chromatograms indicate a well defined difference between white and blue flowers. Results from controlled breeding indicate without doubt that blue is dominant and that individuals are self-incompatible. But they also suggest that two complementary factors may be involved. We have been unable to obtain conclusive evidence of this after numerous trials because of the difficulty of maturing seeds in the coastal climate and because of an inherent property of the seeds, which delays their germination in nature sometimes for many years.

The data we will present in this paper give no evidence of selection or of genetic drift, contrary to what was expected from the assumed differences in size of the breeding groups, after 17 yearly cycles during signal changes in the environment and drastic expansions and contractions of the population. We ascribe this static to protracted dormancy of seeds in the soil and their inherently different requirements for germination. One consequence of differential germination is that the mating groups of each year, large and small, are comprised of individuals drawn from a gene pool that is contributed to by individuals matured under various conditions for at least ten yearly cycles. The effective mating group is accordingly much larger and less variable than would be the case if mating each year should be confined to the offspring of individuals produced the year before.

We are indebted to Professor Sewall Wright for his sustained interest and counsel since the first sampling was made

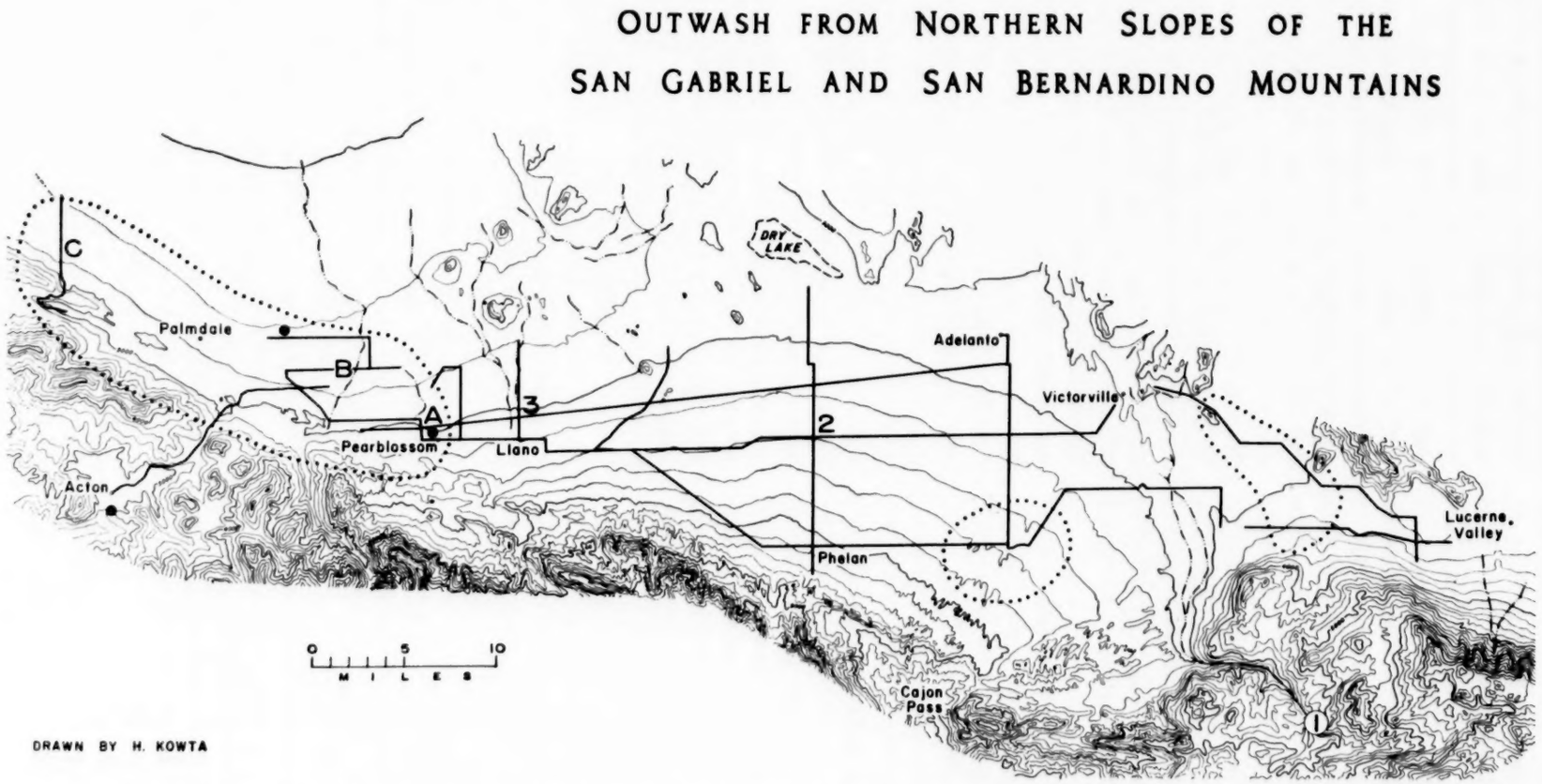

FIG. 1. The area in which *Linanthus parryae* has been studied in 1941 (Epling and Dobzhansky, 1942) and subsequently. The species is found primarily on the outwash indicated by the grid of roadways, and only occasionally at higher elevations to the south or in the more sandy or alkaline areas to the north. A, B and C indicate the positions of one type of sampling station described. The position of the transect referred to is indicated by the dot under A. This dot also indicates the position of a blue homozygous area, as well as those near B and near Acton. The numeral 1 indicates the position of a peripheral mixed area referred to in the text. The numerals 2 and 3 indicate the places where seeds were broadcast.

in 1941 and for his time-consuming analysis of the raw data, as well as to Professor Edward Novitski.

The Habitat

The area where we have studied *Linanthus parryae* lies to the north of the San Gabriel and San Bernardino Mountains along the southwestern margin of the Mojave desert (fig. 1). *Linanthus* and the majority of its associates in this region are confined to the upper gravelly slopes of the alluvial outwash carried northward into the generally dry Mojave River system. Its limits are outlined by the road grid.

The vegetation is a geologically recent community segregated from the Madro-Tertiary Geoflora (Axelrod, 1958). It is dominated by *Larrea divaricata* (the creosote bush) sometimes interspersed with stands of *Yucca brevifolia* (the Joshua tree). The more common smaller shrubs are *Franseria dumosa, Acamptopappus sphaerocephalus, Hymenoclea salsola, Ephedra nevadensis, Grayia spinosa* and *Ericameria cooperi*. Occasionally more transient bunch grasses are found, such as *Oryzopsis hymenoides, Stipa speciosa* and *Hilaria rigida*.

Except at the upper margins of this alluvial plain, the northward gradient is gentle, but sufficient to permit sheet washing during the heavier winter rains. The result is a network of drainages between the bushes that is scarcely noticeable on casual inspection, buttressed by the low mounds around each bush. It is in these shallow flat channels that *Linanthus* and most of its associated annuals are found, genera such as *Oenothera, Canbya, Nama, Cryptantha, Pectocarya, Salvia, Eriophyllum, Malocothrix, Chaenactis, Coreopsis*, and so on. Species for species, *Linanthus* is generally the most abundant.

Annual Cycle

Linanthus parryae is an annual, ordinarily 2–5 cm tall in nature, with a slender stem and branches and small needle-like leaves. Its flowers are relatively large, 1.5–2 cm across. The number per plant is usually 1–4, rarely as many as 10. Its flowering period in nature is limited to a few weeks in April and early May, but is much longer in cultivation, where larger plants may produce as many as 500 flowers. Flowering begins more or less simultaneously for the whole population, so that the flowers of many plants are contemporary. With increasing heat, growth ceases and the seeds mature rapidly, being ready for dispersal in June. The tiny plants remain erect *in situ* until they are beaten down by the winter rains. The mature capsules are partly enclosed by the needle-like calyx lobes and sometimes by the dried corolla. The seeds, which are like fine pepper, are dispersed gradually after the gravelly soil surface has become consolidated by the heat. We have found some seeds in the capsules as late as December. Germination occurs in winter after the first sustained leaching rain when temperatures are lowest. Rains sufficient to wet the soil to several inches depth sometimes occur as early as October when temperatures are generally high, but we have found no germination after them.

Our experimental plants have been grown out of doors in gallon cans. We have scattered the seeds over the soil and have covered them lightly with fine sand, have put them in an open place in autumn, and have allowed them to await the onset of winter rains. This has given the most certain germination. Germination is erratic and differs in amount from year to year and between the offspring of wild individuals. Most plants that germinate grow to maturity. This seems also to be true in nature. The yearly differences in numbers appear to result more from differential germination in response to weather, than from differential survival. Whatever the factors are that control germination, viable seeds persist in the soil for at least ten years. Yet, we have never had seeds germinate after the first year in cans. In years

unfavorable for germination in nature, the plants in flower are few and widely spaced but are usually of normal size and flower number.

Our total experience suggests that for some reason *Linanthus parryae* is tied to an environment at the lower limit of its reproductive potential and persists there, in part at least, because of its faculty for seed storage.

Patterns of Dispersion

The local pattern of dispersion is greatly influenced by the characteristics of the plant community. This remains astonishingly uniform for years in respect of the gross characteristics of soil and the spacing of the perennials. The shrubs that are outlined in figure 2 seem scarcely to have changed in fifteen years. Very few if any new individuals have appeared, and growth is so slow as to be scarcely perceptible. Some of the smaller shrubs have died back in years of drought but have sent out new shoots later and some have perished; but their dead branches remain *in situ* for years. One gets a tremendous sense of stability. The principal indication of change among the perennials is the appearance of an occasional bunch grass, such as *Stipa* in the detritis of the tiny mound that a shrubby *Acamptopappus* once occupied, or the appearance of an occasional short-lived herbaceous *Eriogonum*.

The annuals, on the other hand, like *Linanthus,* may be abundant in one year and absent in another, and variable in density from place to place. In 1941, *Linanthus* formed an almost continuous carpet over hundreds of square miles, resembling a light fall of snow amongst the *Larrea* bushes. In 1950, 1951 and 1959 it was completely absent from the region shown in figure 1 and doubtless elsewhere. Its dispersion, even in good years, is highly variable even over distances of a few feet. This is illustrated in figure 2 which represents two segments of a transect we shall now describe.

This transect was laid out in the spring of 1944 at the place indicated by A on figure 1. It is a half mile long and ten feet wide, and runs from west to east.

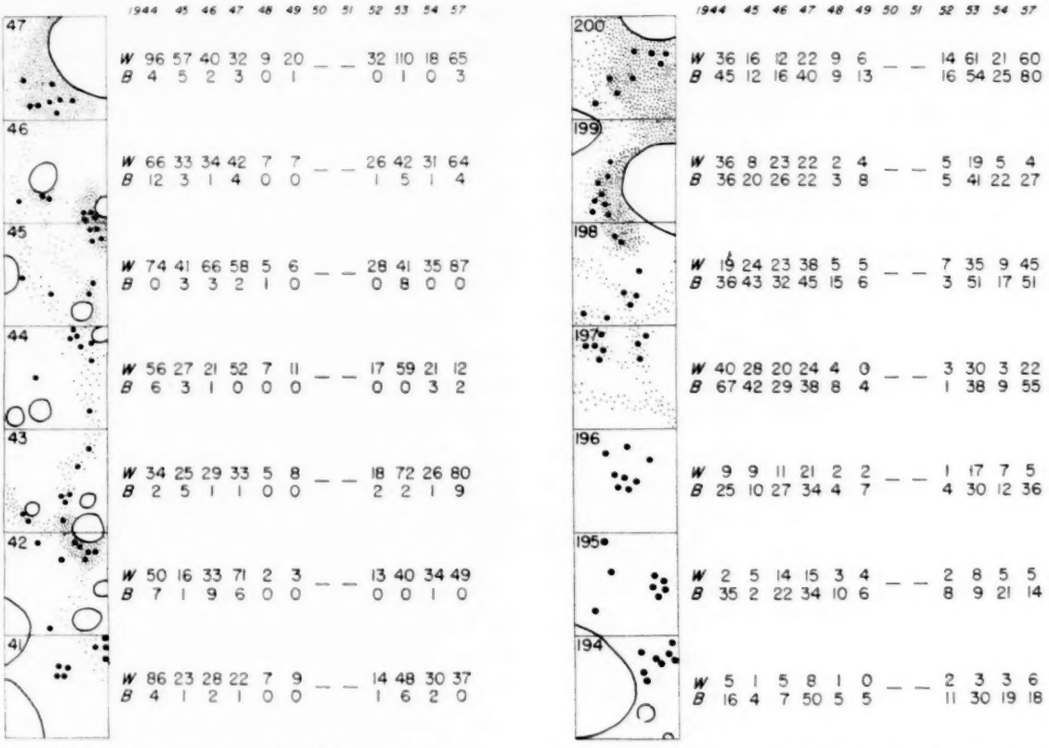

Fig. 2. A chart representing 10 quadrats of an experimental transect. Explanation in the text.

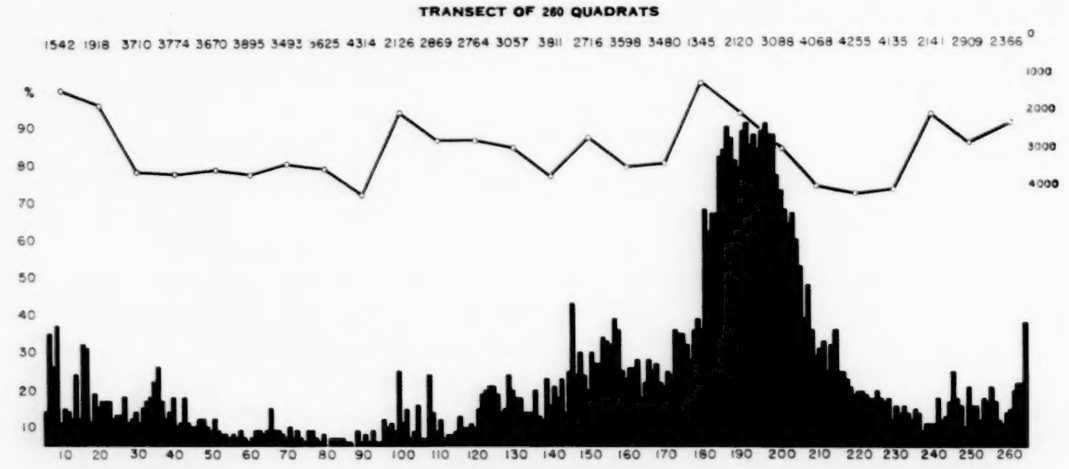

FIG. 3. The mean frequencies for 10 flowering seasons (along the transect described) of blue flowered plants (bar diagram). No plants were found in 1950 and 1951 and very few in 1955 and 1956. Above: numbers of plants per square foot at point of greatest density per quadrat in 10 quadrats for 10 years.

Iron stakes were driven into the ground at intervals of 200 feet parallel to the straight dirt road that services a power line between Pearblossom and Adelanto. The axis of the transect was established by stretching a steel tape between these stakes and its width was established by laying a ten foot surveyor's range pole across the tape at intervals of ten feet. As each quadrat was initially established, the position and spread of the shrubs occupying it were outlined on strips of paper two inches wide on which two inch quadrangles had been marked off and numbered, as shown in figure 2. The limits of each quadrat were readily and accurately identified in each succeeding year by using the tape, the range pole and the position of each kind of shrub.

The fine dots in figure 2 illustrate the areas of greater density of *Linanthus* recorded in 1944. The absence of dots means only that the plants were too diffuse for a pattern to be perceived. The pattern that emerges is a reticulum meandering in the open along the shallow flat drainages between the bushes. Pools of density are found near but not under some bushes. They contract and expand in size and density from year to year, but are usually perceptible. Small clusters of density often appear in the more diffuse quadrats, such as 194. Except for local and yearly fluctuations of this sort, the over-all pattern of densities observed in 1944 has not changed perceptibly after fifteen breeding cycles.

Two methods were used for sampling color frequencies, one on the transect and another on other plots which will be described presently. As we walked along the transect each year, delimiting each quadrat as described above, we put down a ring of wire at the place where the density was greatest. The ring encompassed a square foot. The numbers of blue and white flowered plants within it were counted and recorded at the appropriate place on the work sheets. These places of record are indicated by the larger dots. The places sampled were in fact more clustered than indicated because of limitations of space. Taken as a whole they illustrate the relative stability of the pattern of dispersion. The numbers of blue and white counted each year are shown in the columns on the right. They illustrate the expansion and contraction of the population from year to year and suggest a considerable variation in the size of breeding groups in different quadrats and in different years, which would also depend on the ordinary range of pollen and seed dispersal. Al-

though variable in number from quadrat to quadrat each year, the total number of plants tends to even out over greater distances in the long run. This is shown by the numbers for each series of 10 quadrats given at the top of figure 3 and the curve below.

The densities recorded and the pattern observed indicate that the species is generally adapted to the transect, and indeed to the whole area, but that its abundance is controlled very locally by factors that pertain to germination, even though no consistent differences of habitat are perceptible from quadrat to quadrat.

Mating System

The flowers of *Linanthus parryae* are protandrous and the plants are essentially self-incompatible. Only occasional seeds are obtained by selfing, in contrast to generally full capsules after crossing. The pollen tends to cohere and is of a sort that would not usually be distributed by wind. Each flower remains open for two or three days. The corolla is light responsive and folds loosely around the style and stamens shortly before sundown, opening again after sunrise when the light has reached a certain intensity. The flowering period during which most pollination would be expected lasts about a month, depending on the weather, and reaches its maximum about mid-April. Occasional insects alight on the petals, but the chief, if not the only, pollinator appears to be a species of *Trichochrous* (*Melyridae*). (We are indebted to Professor E. Gorton Linsley for determinations.) This is a small beetle which is generally covered with golden pollen. Alighting on a corolla, it crawls past the stamens and style and stops for a while in the tube. It will crawl out later and fly swiftly to another plant perhaps a foot away, perhaps 10 feet. Its flight pattern is erratic and difficult to follow, but seems to be limited.

Wind seems to be a negligible factor in pollen transfer. During the flowering period, it is regularly on shore and generally parallel with the transect. During the day the breeze is sufficient to cause the plants to nod in an easterly direction, suggesting a transfer along the transect, if any. If wind is a factor one would not expect the persistent differences in color frequencies year after year in adjacent quadrats unless one would invoke extraordinarily strong local selection. The whole data show otherwise.

Total pollination, either of plants or flowers, is by no means completely effected. It is so variable from plant to plant in a restricted locus, and so variable from place to place, and doubtless also from year to year in conjunction with the abundance and activities of the pollinator, that only an approximate estimate of the mean number of seeds per plant is possible. The number of normally developed flowers on a plant that would be receptive to the pollinator, ranges from 1–15. Four random samples of dried plants in fruit were made in 1957, which was a fairly good year. Two samples from all white areas were made at Pearblossom and at Llano, and two from all blue areas were made at Pearblossom and the enclave near Palmdale. The number of flowers on each plant was counted, distinguishing between those that had set seed and those which had not (table 1).

Table 1. *Flower and seed number*

	Pearblossom white	Llano white	Pearblossom blue	Palmdale blue
Sample size	150	150	446	301
Flowers per plant	2.06	4.14	1.64	1.86
Sterile plants (%)	23.5	50.0	34.0	23.5
Seeds per fertile plant	50.2	51.5	30.1	30.1

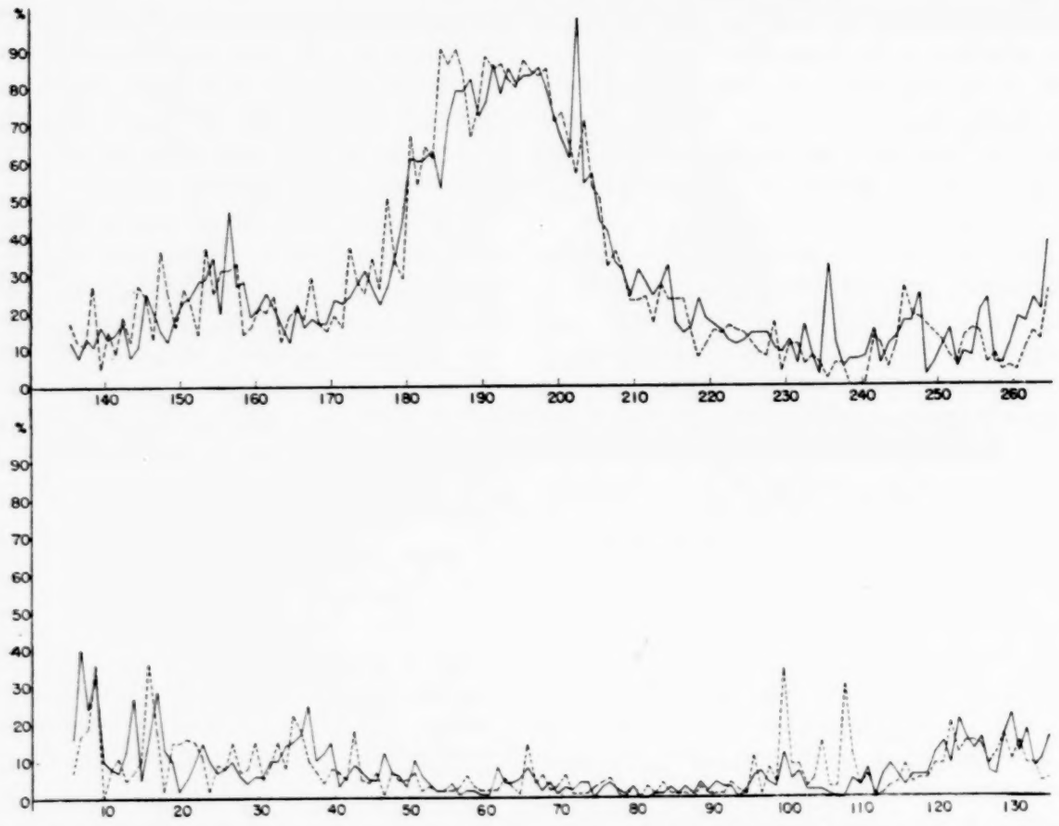

FIG. 4. Comparison of mean frequencies of blue flowered plants during the periods 1944–1947 (solid line) and 1953–1957 (broken line), omitting 1955 and 1956.

The consistent differences between seeds per plant would, at first glance, suggest a much greater prolificity of the white plants. They disappear, however, if one takes into account the disparities in the numbers of flowers and the proportions of unpollinated plants. These data probably mean that in some places and in some years a much greater seed set will occur in one class than in the other. What it would be in blue heterozygotes we have no means of knowing, inasmuch as we are unable, even with chromatography, to discriminate between them and the blue homozygotes. Taking into account the whole data we have used the datum of 30 seeds per plant as the long term average. Its validity can be tested only after systematic sampling.

PHENOTYPIC FREQUENCIES

The mixed areas outlined in figure 1 are the same as those shown in figures 1 and 2 of Epling and Dobzhansky (1942). 427 samples were made during that survey, roughly 160,000 plants having been counted. About 60% were all white (stations 48-102, 233-261, 293-340). No blues were found for 24 miles along the straight road from 293-340, nor along the bisecting road from 355-365. Only an occasional blue or a small cluster were observed elsewhere in the white areas. For example, 23 blues were found among 40,000 plants in samples 29-134. The all blue counts formed not more than 1% of the total count. The mixed areas, however, are far from uniform, almost pure white occurring in some parts, variable proportions of blue and white in others, with occasional all blue enclaves of limited extent.

Forty-four soil samples were made throughout the area from Palmdale to Lucerne Valley and the frequencies of associated shrubs were recorded at each

site. We are indebted to Prof. Martin Huberty for an analysis of the former and to Dr. Harriet Bonner for analysis of the latter. Nothing was found in respect of either vegetation or soil to distinguish the mixed areas from the white areas.

Three kinds of permanently marked experimental plots were set up in 1944: the transect, already described; three stations evenly placed in the western mixed area; and two elimination plots. Their positions are shown in figure 1 at A, B, and C.

We shall first consider the frequencies of blue and white along the transect. The bar diagram in figure 3 gives the mean frequencies of blue for each quadrat from 1944-57. *Linanthus* was completely absent in 1950 and 1951 and the numbers were so small in 1955, 1956 and 1958 as to discourage the effort of recording them. The data therefore pertain to 10 effective years during a period of 15 growing seasons. The total sample consisted of 80,781 plants.

The transect is oriented from west to east and is situated on the margin of the mixed area. Had it been projected eastward somewhat farther it would then have entered the pure white area that extends for miles. Had the western end of the transect been swung southward somewhat, it would have terminated in an all blue area, as we learned later. This is to say that an ovoid area is present near the transect of which the blue area shown is the northern extension, tapering quickly into an almost white area. The cline of frequencies illustrated has remained constant. The mean frequencies of blue flowered plants for each quadrat from 1944–47 are graphed by the solid line in figure 4 and those from 1953–57 by the broken line. Significant deviations have occurred in some quadrats but have not persisted. Great fluctuations of weather and density have occurred meanwhile.

The stability of the all blue homozygous enclaves is no less remarkable, grading abruptly as they do into mixed areas. In addition to the one already mentioned, and an isolated colony found near Acton, another was found in 1947 about 3 miles east of Palmdale, marked by the dot northwest of B (figure 1). In 1947 it consisted of an isolated patch of about 10,000 plants, of which 3 were white. Ten years later, in 1957, *Linanthus* was fairly abundant in and around this area, which had been permanently marked. The all blue enclave was greater in extent than before, occupying perhaps an acre, but the plants were less dense. No whites were found except at its periphery where it graded rather sharply into a mixed area.

The second method of sampling was done by counting *all* the blue and white plants within an area of 100 square feet, an area comparable to each quadrat on the transect. This was done by permanently marking plots of this size in 1944 at A, B and C. Nine pieces of iron pipe were driven into the ground at each station in a cruciate form, 50 feet apart. Each year, a piece of cord of appropriate length was looped over each stake and was used to circumscribe 100 square feet. The proportion of each circle occluded by shrubs was similar to those of the quadrats, on the average, and the dispersion of the plants varied in a similar

TABLE 2. *Numbers of* Linanthus *per 900 square feet per year*

Year	1944	1945	1946	1947	1948	1949	1952	1953	1954	Total
Station A	5,337	1,799	2,620	6,928	572	629	2,233	7,979	—	28,097
Station B	9,560	1,970	1,961	3,092	572	243	514	2,779	3,410	24,101
Station C	3,471	1,535	1,643	4,128	44	490	—	—	—	11,311
Total	18,368	5,304	6,224	14,148	1,188	1,362	2,747	10,758	3,410	63,509

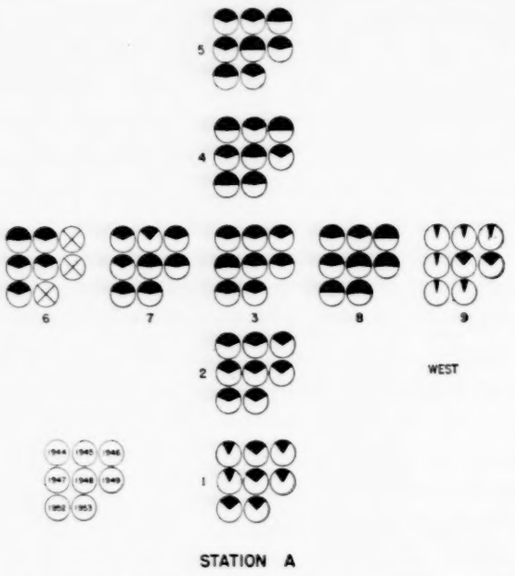

FIG. 5. Relative frequencies of blue (solid area) and white flowered plants at Station A for the years indicated by the key at the lower left.

way. Station C was lost by the invasion of a trailer court in 1950 and Station B was partially destroyed by road construction in 1955. So far as recorded, the data from these stations are not significantly different from those of Station A except in over-all relation frequencies of blue and white. The total counts recorded are given in table 2.

The relative frequencies of blue and white for Station A each year from 1944 to 1953 are shown in figure 5. The reader will recall that no plants appeared in 1950 and 1951. The limited area sampled by these stations appears to be uniform, but it is apparent that the frequencies of blue at plots 1 and 9 are significantly different from those of the others. Although significantly different frequencies were found on some plots in

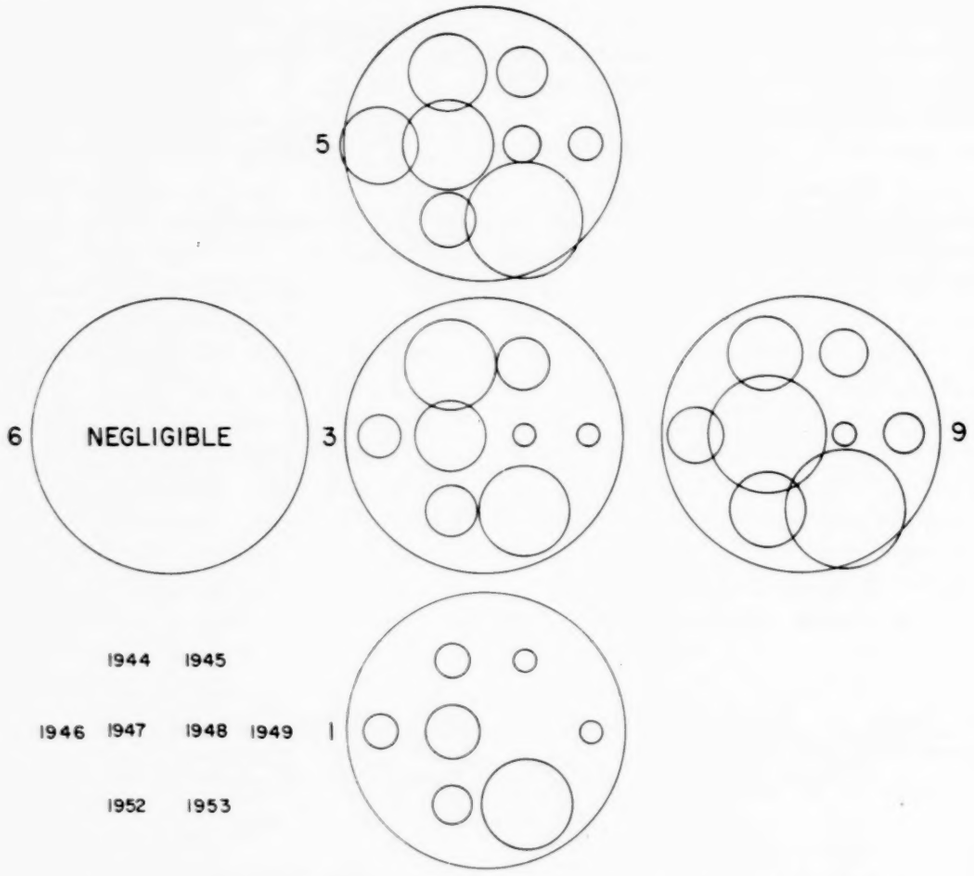

FIG. 6. Relative densities in the years indicated at five of the plots diagrammed in figure 5.

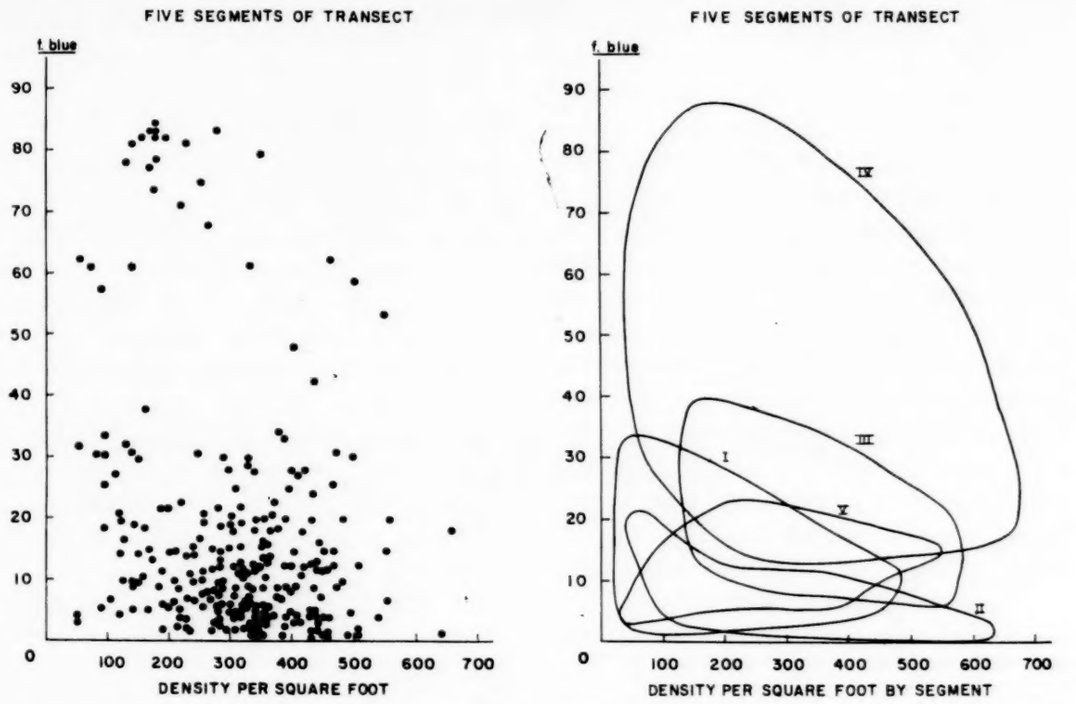

FIG. 7. Left: a scattergram of frequencies of blue flowered plants (ordinate) and the densities per square foot where each sample was recorded, for the whole transect for 10 years. Right: the outlines of the same for each of five segments of the transect.

different years, the usual frequency was restored later. On the whole, the frequencies in each plot remained much the same despite the expansion or contraction of the population and its dispersion from year to year, as indicated in table 2 and figure 6.

This lack of correlation between frequencies of color and density even in very local populations is more clearly demonstrated in figures 7, 8 and 9. In making the work sheet of figure 7 the transect was divided into five segments of approximately equal length. The scatter diagram on the left represents the positions of the samples in all the quadrats. Those of each segment were recorded in a different color. The ovals at the right represent the limits of those in each segment, according to the color used. No correlation was found between the frequencies of blue and the density of the flowering population, measured in this case within the densest square foot, either over the whole transect, or its parts, which vary in the frequencies of blue from

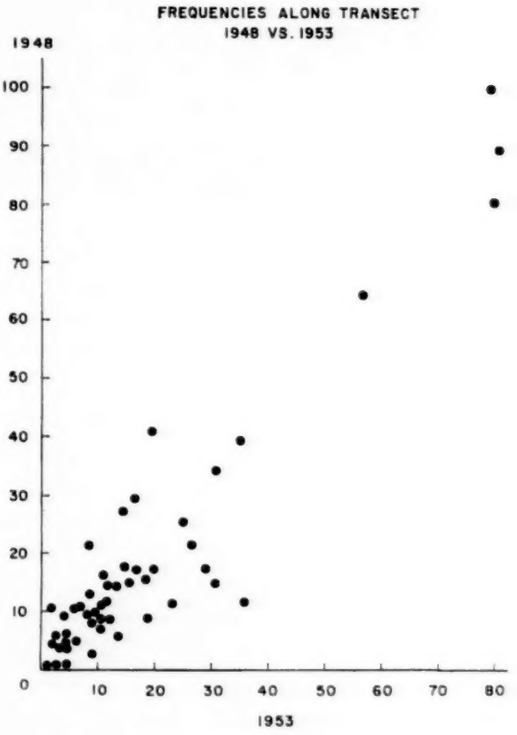

FIG. 8. Comparison of frequencies of blue flowered plants along the transect in 1948, a year of low densities, and 1953, when densities were high.

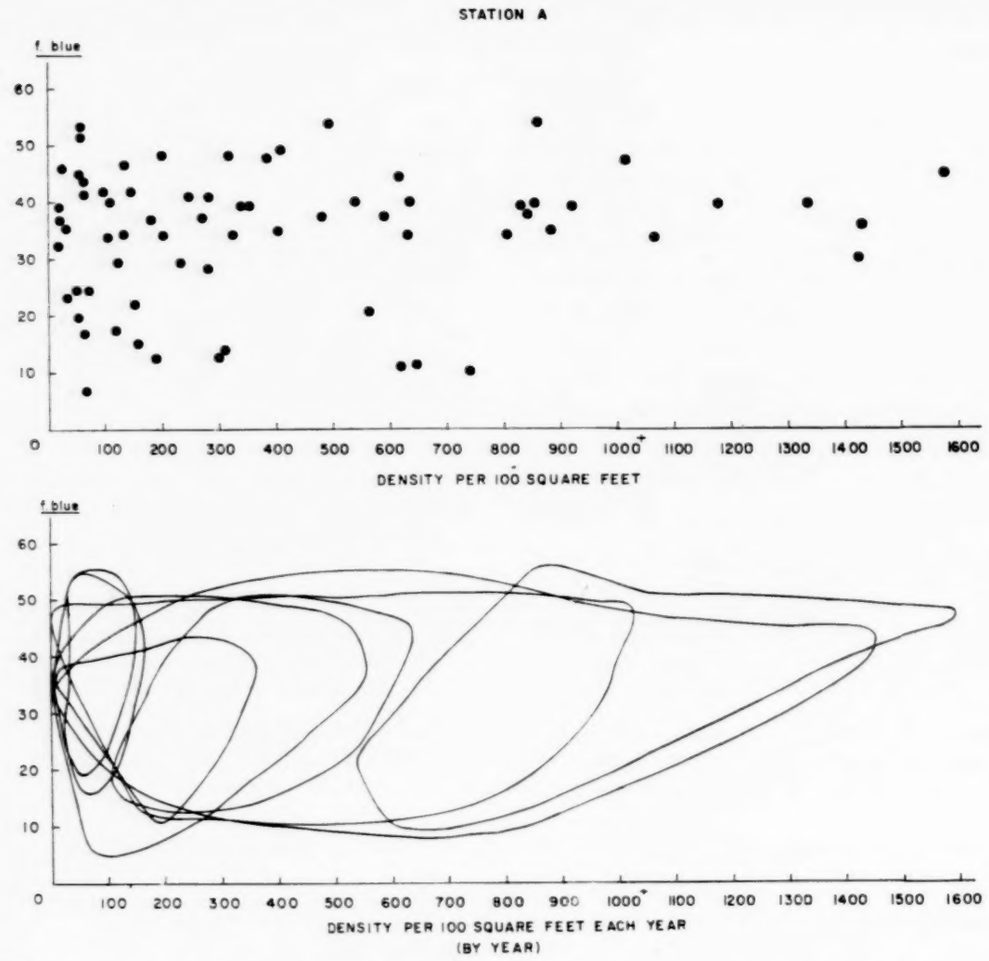

FIG. 9. Above: a scattergram of frequencies of blue flowered plants at Station A from 1944–1953, compared with densities. Below: the same, outlined by year.

almost none to 80% or more. These results were tested further by comparing the frequencies of blue in the years 1948 and 1953. The populations of 1948 were small, those of 1953 were large (figs. 2 and 6). Nevertheless, the relative frequencies of blue in these years were substantially the same. When frequencies and densities at Station A are compared (fig. 9), no correlation is found, either in toto or by year (the ovals), even though much larger numbers of plants were counted in each plot than in the quadrats of the transect.

The third experiment was set up in 1948. It consisted of two elimination plots a few yards from stations A and B respectively, each of which consisted of 3 subplots 50 feet apart, similarly devised to those of the stations A, B, C. The total number of plants was counted in each subplot each year, whereupon all were carefully pulled up while still in flower, and discarded. Thus, each yearly population of a circle was primarily if not wholly derived from seeds already deposited. The upper numbers in the small circles in figure 10 give the *frequencies* of blue; the lower, the *total number* of plants each year. The frequencies of blue did not change substantially from year to year despite large changes in density. Furthermore, the plots show that seeds may remain dormant in the soil for at least seven years and that the number that germinate is variable each year. The results of this experiment are commensurate with our observations of the all-blue

area near Palmdale after a lapse of ten years when no plants appeared.

Transplant Experiments

In November 1954, 20,000 seeds that had been harvested earlier that year from blue plants in an all-blue area near Acton were broadcast in an area remote from blue flowered plants (fig. 1) and the place was carefully marked. The seeds were thoroughly mixed with fine sand from the locality and the mixture was tossed into the air in such a way as to cover roughly 200 square feet in an open area adjacent to a large *Larrea* bush. Only white flowered plants were growing there and in the surrounding territory. When this plot was visited in April, 1958, 23 blue flowered plants were found within the area, together with approximately the same number of whites. All were more or less evenly dispersed. Careful search throughout the surrounding territory found no blue plants at all. Although relatively sparse, the existing plants were vigorous and normal. There were no perceptible differences in vigor between the blues and the white; both carried 2–4 flowers each. Nor was there evidence of migration beyond the area in which blue had been sown. This experiment shows beyond reasonable doubt that a pool of blue flowered individuals from some distance can survive for at least four years within a white territory. Some of these blues were doubtless heterozygotes. The widespread area of the white homozygotes in which only occasional blues are found, suggest that the blues may be eliminated in the long course of time.

Other reciprocal plantings have been made in which similar reciprocal germination has regularly occurred, but the data are not yet sufficient for analysis.

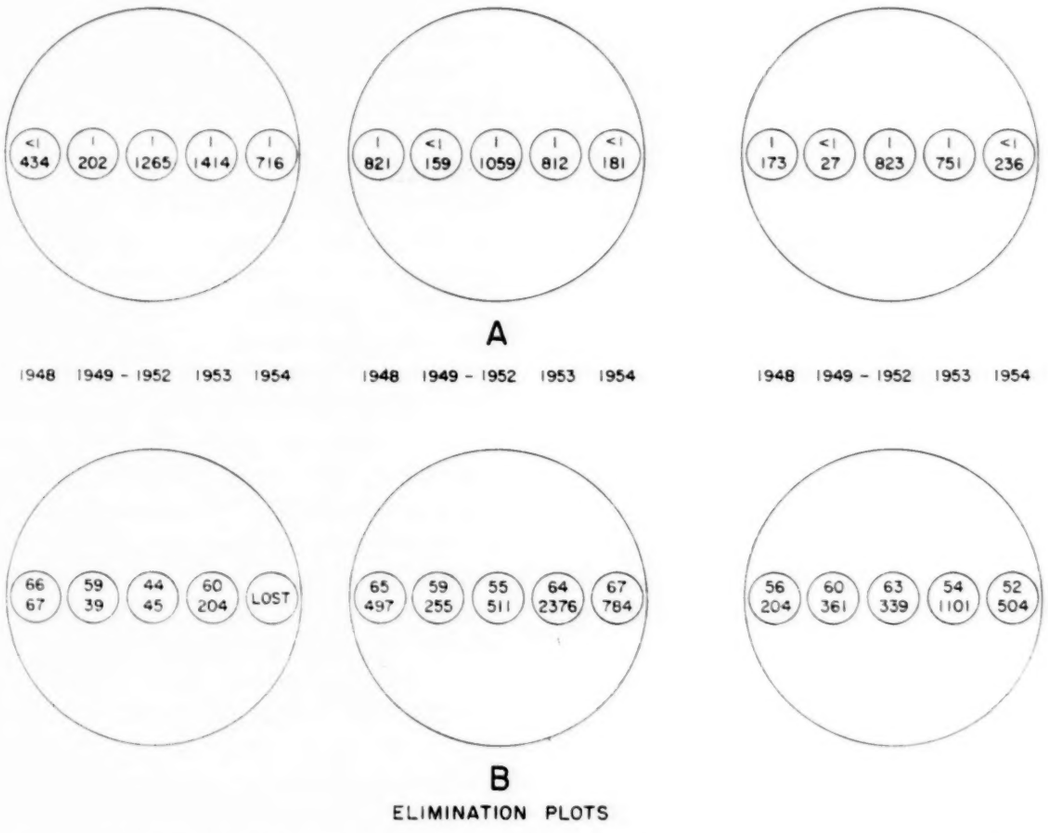

Fig. 10. Elimination plots. Above in each small circle: frequencies of blue flowered plants in the years indicated; below: total number of blue and white plants counted.

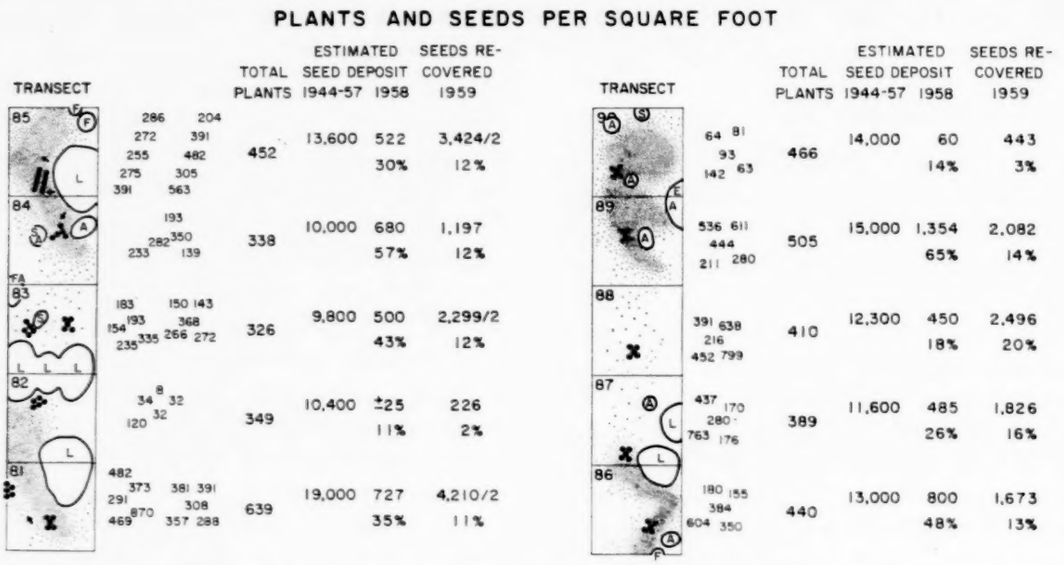

FIG. 11. Quadrats 81–90 of the transect showing positions of seed samples and the resultant data. See text.

THE SEED POPULATION

The data from the controlled elimination plots and our observations of the blue area near Palmdale, show that seeds remain viable in the soil for 7–10 years, and perhaps for much longer. There is no indication that the seed reservoir in any of the elimination plots (fig. 10) was nearing depletion after five seasons of active growth, and after as many as 4,400 plants had appeared in one plot.

Soil samples to determine seed storage were accordingly made in ten quadrats in the segment of the transect where the greatest number of plants had been counted, quadrats 81–90 (fig. 3, 11). The approximate place on each quadrat was determined by our records where the densities had been greatest during the 10 year period, and the plants counted. The small dots indicate the over-all pattern of density in 1944 as in figure 2. Five circlets of stainless steel were constructed about 6 inches in diameter and 2 inches deep. Together, they enclosed an area of about one square foot. These were pressed into the soil at the appropriate place, about 2 inches apart, as shown in figure 11. Two samples were made in quadrats 81, 83 and 85, situated according to the scarcely perceptible drainage gradients, indicated by the arrows. Although each sample included only 1 square foot of surface, the spacing of the circlets, we supposed, would actually sample the mean position where greatest densities had been recorded.

When the circlets had been pressed down even with the surface, three layers of soil were removed from each at approximate depths of 1, 2 and 3 cm. Each layer was later submitted to a screening process in soil screens of proper mesh, using water, in order to remove as much detritis as possible. The residues were dried and examined in detail under a dissecting microscope. The seeds were separated and packeted according to species. By far the greatest number were *Linanthus*, the total number recovered being nearly 20,000. The numbers of the latter are recorded on the right of the transect diagram (fig. 11). 90–95% were found in the top layer. The totals recovered are shown in the fifth column. After the circlets had been pressed into position, the plants of 1958 which had grown in each were removed and the numbers of seeds they had contained were estimated by examining the empty capsules. These estimates are shown in the fourth column. An estimate was made

of the number of seeds deposited during the 10 year period and is shown in the third column. It was obtained by multiplying the number of plants recorded in each quadrat from 1944–57 at the points of greater density (second column) by 30, our estimate of the mean number of seeds to be expected per plant.

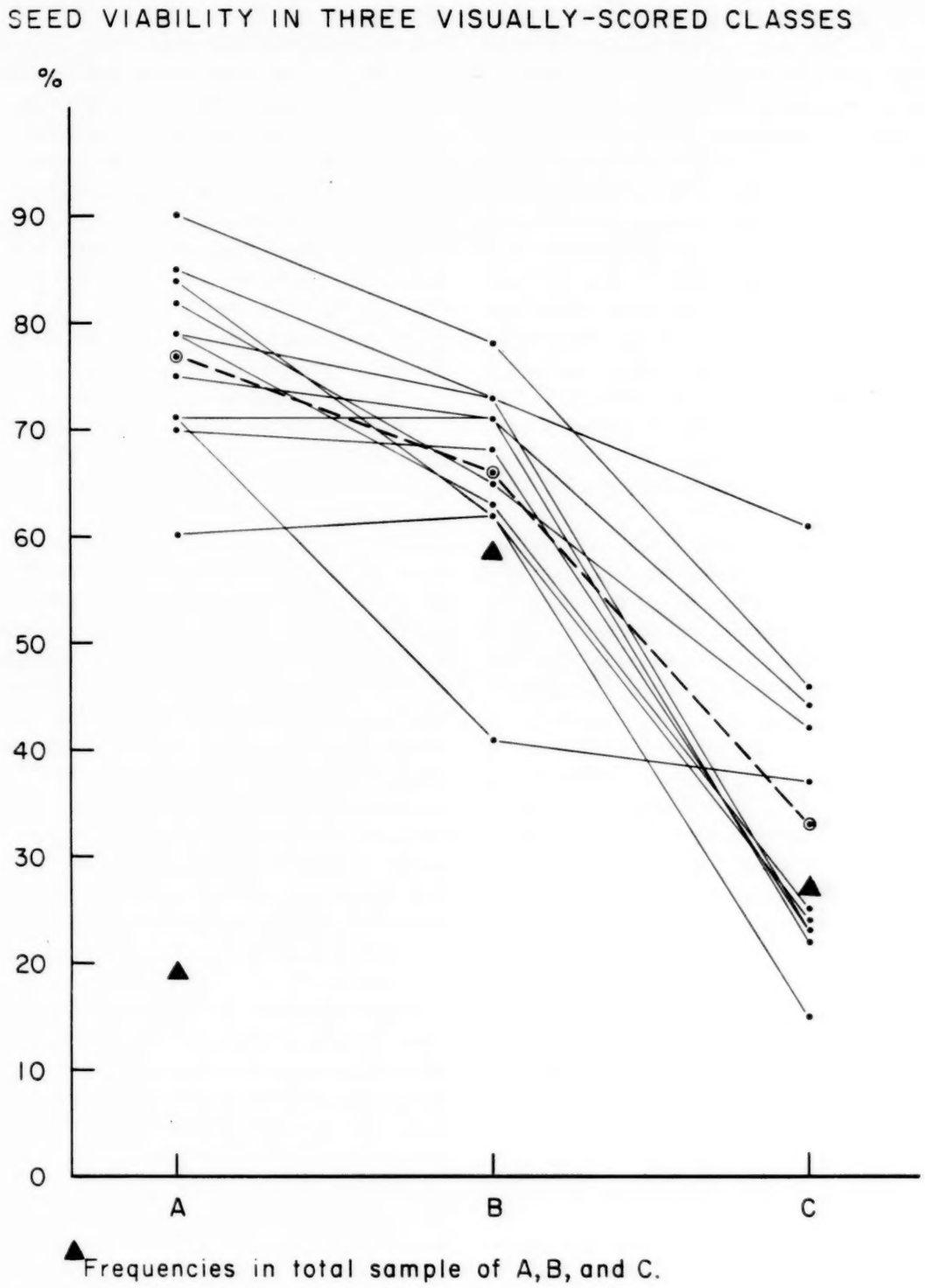

FIG. 12. Percent of viable seeds in each of three classes (A, B and C) and the frequencies of these classes in the whole sample.

When the soil samples had been thoroughly searched, some of them repeatedly, the seeds were classified according to appearance in order to get an estimate of their relative ages. Newly matured seeds are plump, slightly three angled, with a narrow membranous flange along each angle, and of a certain color. Those recovered from the soil had a considerable range of appearance. Some were not very different from fresh seeds, most were obviously weathered, being darker and less fresh in appearance and the walls were more or less sunken into longitudinal sulci. We found it possible to separate them into three approximate classes. Having done this, samples of 100 of each class from each quadrat were tested for viability, using triphenyl tetrazolium chloride under uniform conditions. We are obliged to Professor T. A. Geissman for counsel in this procedure.

The results of these tests are given in figure 12. The circles indicate the percentage of each class in all the samples; the curves indicate the percentages of each class tested in 11 samples. Recalling the subjectiveness of our classification, it seems that roughly 80% of Class A, which constitutes perhaps 20% of the seed population are viable, about 60% of Class B, which constitutes about 25% of the population are viable. Taken together, the data suggest that perhaps 70% of the seeds recovered were still viable, but not equally so in view of their different ages. The addition of seeds to the reservoir each year, particularly in years of great abundance, would tend to maintain the equilibrium of viable seeds, so that many years of unfavorable conditions would need elapse before the reservoir was depleted. This period would be at least 10 years, in view of the data from the elimination plots and may be much longer.

The percentages shown in the fourth column of figure 11 indicate the proportion of seeds contributed to the number recovered. The estimate is probably lower than the norm, because the population was sparse in 1958. The percentages shown in the fifth column give the proportions recovered to those expected from the numbers of plants observed. We might estimate the mean at 30–40%. Our estimate of 30 seeds per average plant may be too high, particularly if large numbers of plants are not pollinated in some places, or perhaps generally in some years when the population of the pollinator is not commensurate with that of *Linanthus*. It may therefore be that a greater proportion of plants are pollinated in years when relatively few plants flower, than in years when they are abundant. Nevertheless, should we decrease our factor of 30 to 15, the proportion of seeds recovered (60–80%) would still fall considerably short of the number expected.

With the onset of hot weather in May and June, *Linanthus* matures and becomes dry and rigid. Most plants remain erect *in situ,* but may sometimes be broken off and lodged against a nearby bush. The upright calyx lobes surround the capsule, and the withered corolla often adheres to it. Some capsules open widely and thus drop their seeds simultaneously. Most open only toward the apex. The seeds are partly retained for at least several weeks and we have found capsules containing a few seeds as late as the autumn rains. When the seeds drop, they fall onto a baked consolidated soil surface and doubtless sift into its minute but scarcely perceptible interstices.

What happens to these seeds thereafter? Some may be eaten by small animals in the soil, but we have found no evidence of this, although empty seed coats are present in the soil. Most are probably eaten by surface animals. Harvester ants are common and are in fact almost the only insect seen during the summer months when the seeds are being deposited. They appear to be primarily concerned with the ubiquitous *Erodium*. We have examined the margins of their

hills and have found no *Linanthus* plants around them. Whether they would gather the minute seeds of *Linanthus* is doubtful.

Wind would appear to be the most effective agent of dispersal. During summer, desert devils of several yards circumference, reaching several hundred feet into the air, are frequent. Their force is sufficient to carry leaves and light detritis with them and it would be surprising if they did not also carry *Linanthus* seeds either from the capsules or from the ground. The distances they travel before they dissipate are generally as long as the transect or longer. Yet, their paths are usually from west to east, just as the sustained breeze of the flowering months, parallel with the transect, but more erratic in direction. It is possible that the occasional patches of blue plants found in the white area to the east can be accounted for on this ground. But it would appear that this method of dispersal is of negligible importance over the long period.

Gales frequently occur during the driest season in autumn before rain has fallen. Their effect on dispersal is also doubtful. For example, we visited our experimental area in 1958 during a gale that was carrying clouds of dust from the cultivated areas north of the grid shown in figure 1 several thousand feet above the mountains to the south. Its gusts would whip the *Larrea* branches almost to the ground. But at the surface all was quiet. *Linanthus* plants still bearing seeds were abundant enough but were scarcely being shaken by the wind. The chief movement of air was indicated by a desultory shifting about of the fuzzy *Larrea* seeds until they would lodge against a stick a few inches away, only to move to another after another gust. The presence of peripheral colonies such as that at Acton or near Lake Arrowhead (fig. 1) might be attributed to such gales. But, they might also be relictual from former distributions, as is suggested by the presence of an all blue area between Shandon and Edna, San Louis Obispo County. Should wind of any kind be a controlling factor in the dispersal of these seeds, one cannot account for the persistent differences of frequency from quadrat to quadrat, unless one postulates very local selection over the long term.

There is still another possible means of seed transport, that is, by sheet washing during the heavy sustained rains of winter. The gradient of the outwash from south to north (fig. 1) is gentle and is often scarcely perceptible. Precipitation may form a thin sheet or film of flowing water sufficient to move soil particles with it and presumably the minute seeds of *Linanthus,* according to the lay of the land. This may result in local transport and may account in part for the "pools" of density near some bushes and the dispersion in more open quadrats. It may also tend to bury seeds.

The replicated samples shown in figure 11 were made along slight surface gradients to test this possibility. The most that can be said is that, if seed transport is accomplished in this way, movement is slight and important only as the result of unusual conditions.

Summary Conclusions

Linanthus parryae is a diminutive annual of the Mojave Desert. Its abundance in a given year results from components of the weather that cannot be specified. In some years it may be so abundant as to form an almost continuous carpet of color between the bushes in the area described, giving the species the common name of Desert Snow. In some years it may be completely absent, and may remain so locally for at least ten years. The dispersion of individuals varies accordingly. When abundant they may have a density of 100 or more per square foot in some places. Within any year their dispersion is not uniform but follows a very local pattern of greater or less concentration. This pattern has persisted during the period of study.

The flowers are either white or blue.

Each plant is essentially self-incompatible. Most plants are white, as the common name suggests, and in many crosses white behaves as a simple recessive, with blue dominant. In some instances, however, blue individuals have appeared in the progenies of white crossed with white in frequencies that suggest the possibility that two complementary loci determine the expression of blue. These seem not to have been due to contamination. Until this problem is solved we are not able to estimate the frequencies of blue or white alleles in mixed or white areas.

The frequencies of the blue and white phenotypes have remained remarkably stable within distances of a few feet during 15 years of systematic observation, despite remarkable expansions and contractions of the species population. The data suggest, first, that if genetic drift has played a role, it has been of only local consequence and not persistent in its effects. They suggest, conversely, an intense local selection because the blues are concentrated in certain areas and because persisting clines of blue and white frequencies have been found. But if selection is the determining factor, it cannot operate at a high intensity and certainly not during each growing season. This is clearly indicated by the fact that transplanted seeds of one color can germinate, develop normally, and presumably set viable seeds in the area of the other. The pattern must be the product, therefore, of selection operating at relatively low intensity or intermittently, augmented by the factors that limit dispersion.

The factors of gene dispersal, either by pollen or by seeds, have been examined. Both indicate a remarkable localization. It would appear that both pollen and seeds are dispersed very locally on the average. This localization is further augmented by seed storage in the soil inasmuch as some seeds, and perhaps many, remain viable in the soil for at least ten years and perhaps longer. Because of this habit, *Linanthus parryae*, although an annual, simulates the mating systems of many long-lived perennials.

Germination is differential among stored seeds, apparently in response to weather. This may arise from any combination of three causes. Some genotypes may respond to one concommitance of the factors of weather and some to another with respect to the very local environment of the species. Or it may be that some genotypes require a certain threshold of maturation in the soil before they germinate. It may also rest on an extremely fine division of habitats, such as a position under the margin of a bush, with its consequences of modifying rainfall and insolation, in contrast to a position two feet away in one of the shallow drainage channels described, or on a "plateau" between bushes that may have no more than an inch of elevation compared to a nearby channel.

Whatever the factors may be that result in seed storage and differential germination, the consequence is to produce an effective breeding group in each locale that is much greater than would be suspected from the impressions gained by observation of the expanding and contracting populations. Its size would differ from place to place and from year to year, together with the abundance and activities of the pollinators and the vagaries of weather. Our data suggest that the total population is sufficiently larger than the annual increment, on the average, that local deviations quickly subside to the norm, like a flattened wave.

The conclusion seems warranted, therefore, that the frequencies of blue and white flowered plants are in the long run the product of selection operating at an intensity we have been unable to measure; and that the large size of the effective population, and the localized dispersion of pollen and seeds, has precluded significant changes in pattern during 15 seasons.

Dimorphism within populations is often explainable on the basis of a selective advantage of heterozygotes. In the pres-

ent instance, the occurrence of areas of phenotypic uniformity and adjacent mixed areas suggests that the pattern may be maintained by selection resulting in homozygosity in the uniform areas and that the mixed areas between may be a consequence of hybridization rather than an adaptive property of heterozygotes.

Acknowledgments

In addition to the colleagues referred to above, we are indebted to numerous associates who have generously helped from time to time with recording the frequencies; and to Mrs. Harlan Lewis and Prof. Mildred Mathias for assistance with pollination.

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THE CYTOPLASMIC "SEX-RATIO" CONDITION IN *DROSOPHILA*

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1. INTRODUCTION

Several instances are known in different Drosophila species in which the sex-ratio sharply deviates from the usual 1:1 relation. Such a species is said to carry a "sex-ratio" condition. In some of these cases, the very unequal proportion of males and females is caused by the action of chromosomal genes. In others, however, this "sex-ratio" condition is transmitted solely through the female parent (Magni, 1952, Cavalcanti and Falcao, 1954, Malogolowkin and Poulson, 1957, Malogolowkin, Poulson and Wright, 1959) and remains constant through a large number of backcrosses to males of other strains (Magni, 1954). In the latter case, the inheritance is regarded as cytoplasmic.

The cytoplasmic condition seems to be due to the presence in the ova of "sex-ratio" females of self-reproducing cytoplasmic particles which may be transmitted to normal females by injection of the contents of abnormal embryos (Malogolowkin and Poulson, 1957, 1958). The effect of this cytoplasmic condition is that the males derived from "sex-ratio" mothers die, mostly in the embryonic stage, leaving only few if any survivors, so that e.g. only about 0.5 per cent of the adults produced by *D. bifasciata* "sex-ratio" females are males. These males are fertile and do not transmit the "sex-ratio" character (Magni, 1952).

The cytoplasmic "sex-ratio" condition may occasionally be lost, by inactivation or loss of the cytoplasmic particles. Magni (1952, 1953, 1954) could inactivate the "sex-ratio" condition in *D. bifasciata* by temperature shocks at a sensitive period. Females derived from such temperature-treated mothers may have offspring with a normal (1:1) sex-ratio or a highly abnormal sex-ratio (99.5:0.5), or intermediate sex-ratios, and continue to give this result in succeeding generations. Occasionally, female offspring from a heat-treated female will give rise to normal offspring only (Magni, 1953). Heat treatment does not produce, however, a similar effect in *D. willistoni* (Malogolowkin, 1958). Furthermore, nuclear genes have been described (Cavalcanti, Falcao and Castro, 1957, 1958, Malogolowkin and Poulson, 1958, Malogolowkin, 1958) which inhibit the multiplication of the cytoplasmic particles, so that "sex-ratio" females containing these genes give normal sex-ratios and their daughters also give normal sex-ratios. "Sex-ratio" cytoplasm may, then, "mutate" to the normal state under the influence of genes and of environmental conditions.

All "sex-ratio" strains have been established from females caught in the wild. In many cases (Magni, 1952, Malogolowkin, 1958) a small number of individuals show this condition, but occasionally, a high proportion of the females in a local population may be affected (25%, Cavalcanti and Falcao, 1954). Magni (1958) has presented evidence from experiments using population cages that "sex-ratio" cytoplasm may have a selective advantage over normal cytoplasm in females.

The present paper attempts to investigate the conditions which control the persistence and frequency of a "sex-ratio" cytoplasm and of interacting genes in a population, particularly the

[1] Prepared in connection with research sponsored by the Office of Naval Research.

effect of selective advantage. In Section 2, the case of variable selective values in the absence of interacting genes is considered. In Section 3, the effect of interacting genes under constant selective values is studied.

2. Simple Model

In the model to be formulated in this section, no account is taken of genes which support or inhibit the multiplication of cytoplasmic particles. The problem of genic interaction will be dealt with in Section 3. It is first necessary, as a prerequisite for further study, to formulate the simpler model. This will make possible a detailed study of the consequences of the selective advantage of "sex-ratio" cytoplasm over normal cytoplasm, noted by Magni (1958).

It will be assumed that all mating is random and that the cytoplasm of the offspring is identical with that of the maternal parent with the exception that a fraction of the eggs laid by "sex-ratio" females is normal i.e. the same as eggs laid by normal females. This is the same as saying that the cytoplasmic particles in a female have only a chance $1 - t$ of being passed on to her offspring. Since males pass on no cytoplasm, they need not be considered so we may describe the mating population by

α = fraction of females who carry normal cytoplasm,
β = fraction of females who carry "sex-ratio cytoplasm,

where $\alpha + \beta = 1$. If their egglaying capabilities are equal, then the ratio of normal to "sex-ratio" eggs is as $\alpha + \beta t$ to $\beta(1 - t)$. One half of the normal eggs will yield viable males and one half the "sex-ratio" eggs will yield inviable males, the remainders being viable females. If a fertilized female "sex-ratio" egg has S times more chance of becoming a parent in the next generation than a fertilized female normal egg, where S is a number greater than unity and possibly a function of β, then the relative proportions of

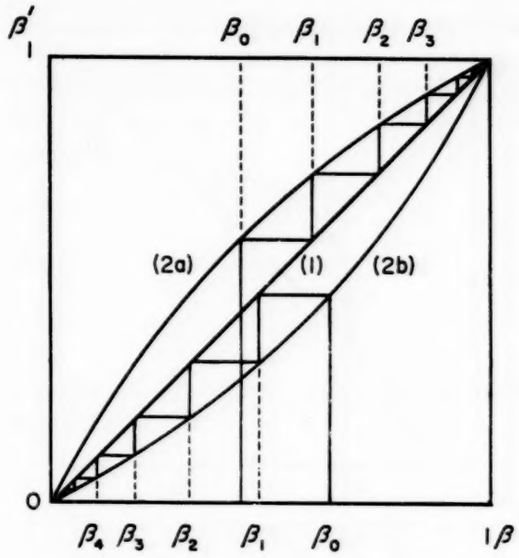

Fig. 1. The relation of "sex-ratio" cytoplasm proportions in successive generations. Curve (1): $\beta' = \beta$. Curve (2a) is the graph of (2.2) for $t = 0$, $S = 2$. Curve (2b) is the graph of (2.2) for $t = 0$, $S = 0.5$.

normal and "sex-ratio" females in the next generation's mating population are, respectively,

$$\alpha' = \frac{\alpha + \beta t}{\alpha + \beta t + S\beta(1 - t)},$$

$$\beta' = \frac{S\beta(1 - t)}{\alpha + \beta t + S\beta(1 - t)}.$$

Recalling that $\alpha = 1 - \beta$ and that $S = S(\beta)$, the behavior of the system can be studied graphically. Equilibrium means that

$$\beta' = \beta. \tag{2.1}$$

However

$$\beta' = \frac{S(\beta)\beta(1 - t)}{1 + \beta(1 - t)[S(\beta) - 1]}. \tag{2.2}$$

Hence if these two relations are plotted as curves of β' against β, equilibrium corresponds to the intersection of these curves. And what is more important, the nature of the equilibrium may easily be deduced from these curves. In figure 1, curve (1) is the diagonal of the square while curve (2) is drawn for two cases, $t = 0$, $S > 1$ (2a) and $t = 0$, $S < 1$.

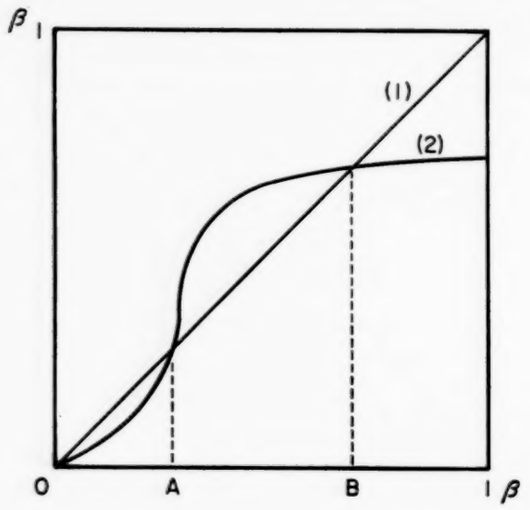

FIG. 2. The relation of "sex-ratio" proportions in successive generations. Curve (2) represents a case where $S(\beta)$ and t are such that both stable (O and B) and unstable (A) equilibrium states exist.

(2b) It is clear that, in Case (a), whatever the initial value of β, ${}_{(a)}\beta_0$ say, the successive use of (2.2) to find new values ${}_{(a)}\beta_1$ ${}_{(a)}\beta_2$ etc. of β is equivalent to the construction indicated in figure 1 and that β tends to unity as the number of generations increases.

The fact that $\beta = 1$ leaves no males in the population means only that it is not possible for S to remain constant under these conditions. In Case (b), β tends to zero. Both these results are intuitively clear.

The curve of (2) always passes through the origin, as it must since $\beta = 0$ is always a possible equilibrium state. From figure 1, we see that in Case (b) the origin is a stable state whereas for Case (a) it is unstable. By various choices of the parameter t and the function $S(\beta)$, the curve (2) can take a wide variety of shapes.

The geometrical construction explained in figure 1 rapidly shows whether the equilibria are stable or not. For example in figure 2, the equilibrium A is unstable whereas equilibrium B is stable. For in this case if the system is started with a β_0 in the interval (O, A), β decreases steadily to zero; if it is started with a β_0 in (A, B), β steadily increases to B; if it is started with β_0 in (B, 1), β decreases steadily to B.

This raises the question of whether the value of β can converge to an equilibrium value in an oscillatory rather than a monotonic fashion. In figure 3, the curve (2) is seen to produce this behavior. A little more experimental curve sketching will give a case where the oscillation may never die down. In both cases, it is necessary that the slope of curve (2), at the point where it cuts curve (1), be negative.

Thus, depending on the nature of the function $S(\beta)$ and the value t, the system may show a wide variety of phenomena. It is now necessary to determine what functions $S(\beta)$ and values t lead to these phenomena. Given a sequence of successive values of β from an experiment β_0, β_1, β_2, etc., points of the appropriate curve (2) may be plotted—they have coordinates (β_0, β_1), (β_1, β_2), etc. If these points are joined by a smooth curve, it should give an approximation to the real curve (2) for the range of β covered. Furthermore the relation (2.2) may be solved for $S(\beta)$ to find

$$(2.3) \qquad S(\beta) = \frac{\beta'(1 - \beta(1 - t))}{(1 - \beta')\beta(1 - t)}$$

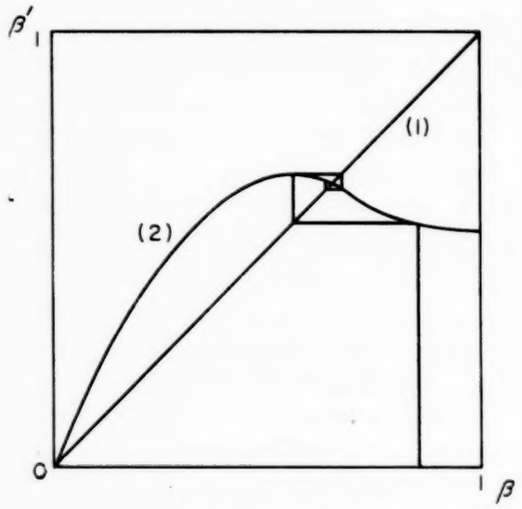

FIG. 3. The relation of "sex-ratio" proportions in successive generations. Curve represents a case where $S(\beta)$ and t are such that there is an oscillating convergence to the equilibrium state.

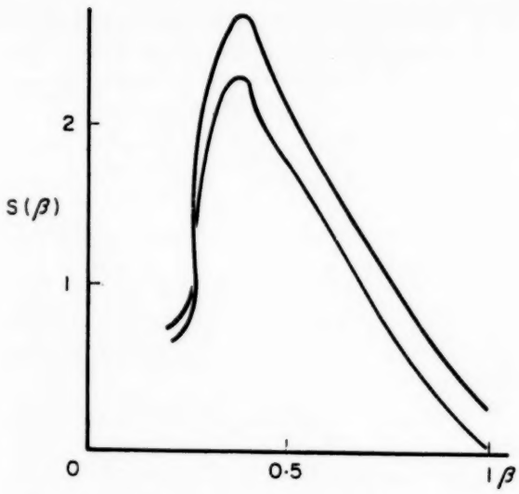

FIG. 4. The dependence of the selection factor on the proportion of the "sex-ratio" cytoplasm. Curves (i) and (ii) show how S must vary with β so that curve (2) of figure 2 is produced when t is respectively 0 and 0.1.

If t is known—for example, from the study of the number of males in the offspring of "sex-ratio" females—the (approximate) graph of $S(\beta)$ against β may be constructed from the (approximate) curve (2). For it is then possible to compute the right-hand-side of (2.3) for various points (β, β') on curve (2). It was in this way that figures 4 and 5 were constructed from, respectively, figures 2 and 3.

A study of these figures suggests that, if $S(\beta)$ is a constant, oscillations about a stable equilibrium state cannot occur. This is easily verified since, if S is constant,

$$\frac{d\beta'}{d\beta} = \frac{S(1-t)}{[1+\beta(1-t)(S-1)]^2} > 0, \text{ all } \beta.$$

Since we have seen that, for oscillations about an equilibrium point, it is necessary for $d\beta'/d\beta$ to be negative, it is clear that such oscillations cannot occur when $S(\beta)$ is constant. A comparison of figures 4 and 5 indicates that S must decrease, from a sufficiently large value, quite rapidly for oscillations to occur. A precise analytical condition for the possibility of oscillations could be derived but hardly seems necessary. Hence, in particular, the oscillations reported by Magni (1958) imply that in his case $S(\beta)$ was not constant.

If S is constant, the solution of (2.1), (2.2) is either $\beta = 0$ or

$$\beta = \frac{S(1-t)-1}{(1-t)(S-1)}, \tag{2.4}$$

i.e.

$$\alpha = \frac{t}{(1-t)(S-1)}. \tag{2.4}$$

It is clear that (2.4) will represent the equilibrium provided

$$S(1-t) > 1,$$

i.e. provided the product of the selective advantage of abnormals and the chance of passing on the cytoplasmic particles is greater than unity.

Finally it is hoped that the graphical method of discussion, which is generally applicable to all problems where a recurrence relation like (2.2) is involved, may prove useful in other contexts.

3. Gene-Cytoplasm Interaction

In this section we wish to examine the population consequences of introducing a gene-cytoplasm interaction into the

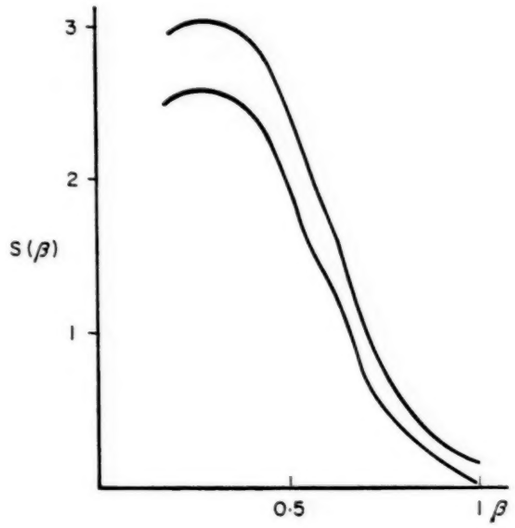

FIG. 5. The dependence of the selection factor on the proportion of the "sex-ratio" cytoplasm. Curves (i) and (ii) show how S must vary with β so that curve (2) of figure 3 is produced when t is respectively 0 and 0.1.

TABLE 1. *Relative proportions of ova formed by females from a population containing normal and "sex-ratio" cytoplasms and alleles SR and sr. (Meaning of symbols explained in text)*

Cytoplasm	Normal		"Sex-ratio"	
Gene	SR	sr	SR	sr
Relative Proportions	$p\alpha + tr\beta$	$q\alpha + ts\beta$	$r\beta(1 - t)$	$s\beta(1 - t)$

model, based on the work of Magni (1958) of Section 2. It will be supposed that a fraction α of the female population carries normal cytoplasm and that a fraction $\beta = 1 - \alpha$ carries "sex-ratio" cytoplasm. Further we will postulate a recessive autosomal gene sr in whose presence, in homozygous condition, "sex-ratio" cytoplasm reverts to normal cytoplasm (Cavalcanti *et al.*, 1958). If, as has been suggested by Malogolowkin and Poulson (1957), and Malogolowkin, Poulson and Wright, (1959), "sex-ratio" cytoplasm carries infective particles, then the SR gene is necessary for their continued multiplication. Let the frequency of SR and the frequency of its allele sr in normal cytoplasm be respectively p and q; let the frequencies of the same genes in "sex-ratio" cytoplasm be r and s. The assumption of equal gene frequencies in males and females with normal cytoplasm is not a restriction since, in our model, it is easy to prove that inequalities are removed in one generation. Further suppose as before that only a fraction $1 - t$ of eggs laid by females with "sex-ratio" cytoplasm carry the "sex-ratio" condition, the remainder t being normal i.e. there is a chance t that no infective particles will be passed into the eggs from an infected mother. Finally it will be supposed as in Section 2 that a fertilized female "sex-ratio" egg has S times more chance of becoming a parent in the next generation than a fertilized female normal egg, where S is a number greater than unity.

If mating is random, it is easy to set up equations relating the genic and cytoplasms proportions in the successive generations. The population described above produces eggs according to table 1, and the sperm are SR and sr in proportions p and q. Recalling that if a zygote receives two sr genes and a "sex-ratio" cytoplasm, it becomes normal cytoplasmically, being homozygous for sr, the relative proportions of adult females in the next generation are

$$\begin{aligned}
\text{normal SR SR} &= p(p\alpha + tr\beta),\\
\text{normal SR sr} &= p(q\alpha + ts\beta) + q(p\alpha + tr\beta),\\
\text{normal, sr sr} &= q(q\alpha + tr\beta) + qs\beta(1 - t),\\
\text{"sex-ratio," SR SR} &= Spr\beta(1 - t),\\
\text{"sex-ratio," SR sr} &= S[ps\beta(1 - t) + qr\beta(1 - t)].
\end{aligned}$$

These proportions add to unity if divided by their sum

$$\alpha + t\beta + S(1 - t)\beta(p + qr) + qs\beta(1 - t).$$

Hence, if α', p', r' denote the values of α, p, r in the next mating generation,

$$\beta' = \frac{\alpha + t\beta + qs\beta(1 - t)}{\alpha + t\beta + S(1 - t)\beta(p + qr) + qs\beta(1 - t)}, \tag{3.1}$$

$$p' = \frac{2p\alpha + pt\beta + tr\beta}{2[\alpha + t\beta + qs\beta(1 - t)]}, \tag{3.2}$$

$$r' = \frac{p + r}{2(p + qr)}. \tag{3.3}$$

We note immediately that if $p = r = 1$ i.e. there are no sr genes, equation (3.1) reduces to

$$\alpha' = \frac{\alpha + t\beta}{\alpha + t\beta + S(1 - t)\beta},$$

as found in the previous section where we studied the effect of allowing S to vary with the value of β. The main result there was that a rapid decrease in S could cause a damped or perpetual oscillation. Here it does not seem possible to discuss a variable selective advantage, except by computing equations (3.1), (3.2), (3.3) repeatedly. (It should be mentioned here that a gene-cytoplasm interaction of the kind discussed here, combined with a variable selective value, would represent a model of the behavior of the well-known kappa particles in *Paramaecium* (Sonneborn, 1947).) This is an easy task for a modern computer such as an I.B.M. 650.

For the case of S constant, the equilibrium values of α, p and r may be considered mathematically. In the case of no sr genes i.e. $p = r = 1$ initially, the previous section showed that the equilibrium value of α is either unity when $S(1 - t) < 1$, or

$$\frac{t}{1 - t}\,\frac{1}{S - 1},$$

when $S(1 - t) > 1$.

To find the general formula from equations (3.1), (3.2), (3.3), we note first that, if we write

$$(3.4)\qquad \begin{cases} A = t + qs(1 - t), \\ B = S(1 - t)(p + qr), \end{cases}$$

equation (1) may be written

$$\alpha' = \frac{\alpha + \beta A}{\alpha + \beta(A + B)}.$$

Thus $\alpha' \gtrless \alpha$ if

$$\alpha + \beta A \gtrless \alpha^2 + \alpha\beta(A + B),$$

i.e. if

$$A \gtrless \alpha(A + B - 1).$$

Hence α chases the current value of

$$(3.5)\qquad \frac{A}{A + B - 1}$$

and, if this tends to a limiting value between zero and unity after many generations, it is the (stable) equilibrium value of α. The ratio (3.5) will be a proper fraction if $B > 1$. The equilibrium equation $\alpha' = \alpha$ has as its solutions unity and the ratio (3.5) so that when this ratio is not in (0, 1), α will tend to unity.

The limiting values of A and B depend on the limiting values of p and r. When the limiting value of α is not unity, the equilibrium equation $p' = p$ becomes

$$(3.6)\qquad 2p[t + qs(1 - t)] = t(p + r).$$

The equilibrium equation $r' = r$ is always

$$(3.7)\qquad 2r(p + qr) = p + r.$$

Thus equations (3.6) and (3.7) are simultaneous equations for p and r. From equation (3.7) it follows that

$$(3.8)\qquad p = \frac{r(2r - 1)}{1 - 2r(1 - r)},$$

$$q = \frac{1 - r}{1 - 2r(1 - r)}.$$

It will be seen that $p = 0$ if $r = \frac{1}{2}$ and $p = 1$ if $r = 1$. Substituting the relations (3.8) into (3.6), we find that r must either be unity or satisfy

$$(3.9)\qquad 2tr^2 - 2r + 1 = 0.$$

When t may be assumed to be small, (3.9) gives

$$(3.10)\qquad r = \frac{1}{2} + \frac{t}{4} + \frac{t^2}{4}.$$

It is clear from (3.3) that always $r \geqslant 1/2$, whatever the value of t. Finally, the use of (3.9) in the formula (3.8) for p reduces it to

$$(3.11)\qquad p = \frac{rt}{1 - t}.$$

To elucidate the limiting behavior of this genetic system, given the initial conditions and the values of t and S, we proceed as follows. Find r from (3.9) (or (3.10)) and p from (3.11). With these values compute A and B from (3.4) and the limiting ratio (3.5) for α. We find that these limits are

$$(3.12) \qquad A = \frac{1}{2}, \quad B = \frac{S}{2}.$$

It is of interest to note that, from (3.4), A is the chance that an egg from an infected mother is normal. The writer is unable to see any direct argument for why this chance should tend to 1/2. Using (3.12), (3.5) becomes

$$(3.13) \qquad \alpha = \frac{1}{S - 1},$$

which is a proper fraction if $S \geq 2$. Thus if $S > 2$ and $r < 1$, the "sex-ratio" cytoplasm is not eliminated and only the fraction (3.13) of females have the normal cytoplasm.

If the alternative solution $r = 1$ is appropriate, $p = 1$, and

$$(3.14) \qquad A = t, \quad B = S(1 - t)$$

so that the ratio (3.5) becomes

$$(3.15) \qquad \frac{t}{1 - t} \frac{1}{S - 1},$$

as in (2.4′).

Since the above analysis turns on the use of (3.6) which requires for its validity that α be less than unity, the procedure is now clear for the case $S > 2$. It is the following:

$$(3.16) \begin{cases} \text{if } 0 < t < 1/2, \text{ r is solution of (3.9) unless initially } r = 1 \text{ when the alternative solution } r = 1 \text{ is relevant,} \\ \text{if } 1/2 \leqslant t < 1, \ r = 1, \\ \text{if } r < 1, \text{ p defined by (3.11), } \alpha \text{ defined by (3.15),} \\ \text{if } r = 1, \ p = 1 \text{ from (3.7) and } \alpha \text{ is defined by the smaller of (3.15) and unity.} \end{cases}$$

The case that remains is $S \leq 2$ and p and r not initially unity. Here α will

TABLE 2. *Values of the proportions of normal cytoplasm (α), of the frequency of the gene SR in normal cytoplasm (p), and of the frequency of the gene SR in "sex-ratio" cytoplasm (r), for different rates of spontaneous loss of cytoplasmic "sex-ratio" particles (t) and different selective values for "sex-ratio" cytoplasm (S) after 100 generations of random breeding. Initial values $\alpha_0 = 0.1$, $\beta_0 = 0.9$, $p_0 = 0.5$, $r_0 = 0.5$*

S	0.5	1	1.5	2	2.1	2.5
t = 0						
g	1	1	1	.9785↑	.9082↑	.6666
p	.1026	.0500	.0150	.0003↓	.00001↓	0
r	.5473	.5239	.5074	.5002↓	.50000↓	.50000
t = 0.05						
α	1	1	1	.9764↑		.666
p	.1597	.1062	.0567	.0279↓		.0271
r	.5713	.5489	.5290	.5736↓		.5732
t = 0.10						
α		1	1	.9734		
p		.1692	.1060	.0606		
r		.5726	.5487	.5288		
t = 0.15						
α		1	1			
p		.2164	.1615			
q		.5943	.5721			

TABLE 3. *Values of the proportions of normal cytoplasm (α), of the frequency of the gene SR in normal cytoplasm (p), and of the frequency of the gene SR in "sex-ratio" cytoplasm (r), for different rates of spontaneous loss of cytoplasmic "sex-ratio" particles (t) and different selective values for "sex-ratio" cytoplasm (S) after 100 generations of random breeding. Initial values $\alpha_0 = 0.5$, $\beta_0 = 0.5$, $p_0 = 0.5$, $r_0 = 0.5$*

S	0.5	1	1.5	2	2.1	2.1
t = 0						
α	1	1	1	.9765↑	.9077↑	.6666
p	.3630	.2500	.0505	.0006↓	.00003↓	0
r	.6521	.6076	.5241	.5005↓	.50001↓	.5000
t = 0.05						
α	1	1	1	.9740↑		.6666
p	.3791	.2971	.1012	.0283↓		.0270
r	.6584	.6262	.5467	.5138↓		.5132
t = 0.10						
α		1	1	.9703↑		
p		.3358	.1614	.0612↓		
r		.6414	.5720	.5290↓		
t = 0.15						
α		1	1			
p		.3668	.2298			
r		.6536	.6996			

tend to unity and the final values of p and r depend on the initial conditions, although they are related still through (3.7). It was not possible to find formulae for p or r so that some examples were followed numerically for 100 generations in an I.B.M. 650. For most of the examples the convergence is very

TABLE 4. *Values of the proportions of normal cytoplasm (α), of the frequency of the gene SR in normal cytoplasm (p), and the frequency of the gene SR in "sex-ratio" cytoplasm (r), for different rates of spontaneous loss of cytoplasmic "sex-ratio" particles (t) and different selective values for "sex-ratio" cytoplasm (S) after 100 generations of random breeding. Initial values $\alpha_0 = 0.9$, $\beta^0 = 0.1$, $p_0 = 0.5$, $r_0 = 0.5$*

S	0.5	1	1.5	2	2.1	2.5
t = 0						
α	1	1	1	.9753↑	.9074↑	.6666
p	.4810	.4500	.0684	.00066↓	.00003↓	.1069
r	.6993	.6867	.5322	.5003↓	.50002↓	.50000
t = 0.05						
α	1	1	1	.9724↑		.666
p	.4827	.4627	.1253	.0284↓		.0271
r	.7000	.6918	.5570	.5739↓		.5732
t = 0.10						
α			1	.9682↑		
p		.4711	.1958	.0614↓		
r		.6952	.5860	.5291↓		
t = 0.15						
α		1	0.9995↑			
p		.4711	.2837			
r		.6977	.6209			

rapid. But those proportions followed by $\uparrow$ (or $\downarrow$) were still increasing (decreasing) at 100 generations. An examination of the tables for $S \geqslant 2$ verifies the mathematical predictions made in (3.16) and in (3.7). Since, when $S \to 0$, the "sex-ratio" flies disappear, p must tend to 0.5, its initial value in tables 2, 3, 4 and this tendency is plainly seen. It is clearer when $\alpha = 0.9$ than when $\alpha = 0.1$ because the "sex-ratio" flies being less numerous, are more quickly eliminated when $\alpha = 0.9$ than when $\alpha = 0.1$. When $\alpha = 1$, the value of r is biologically without interest as it refers to the gene-frequency in a population at the point of extinction of the "sex-ratio" flies. For given S and initial conditions, an increase in t increases p. An increase in t means that there is more chance that the "sex-ratio" factor will be lost in transmission. This should mean that α would tend to unity faster and, since p is that value of p obtaining when α reaches unity, that p is nearer to its initial value i.e. larger—as it is in tables 2, 3, 4. But the tabulated results (not given here) for $S < 2$ and the results for $S = 2$ in tables 2, 3, 4 show that the convergence is actually slower for larger t. The true explanation is that when the normal and "sex-ratio" populations are first put together p drops because of the influx of "sex-ratio" individuals who have become normal because of the segregating of sr-sr homozygotes and the effect is seen to be greater when $\beta = 0.9$ than where $\beta = 0.1$ as would be expected. An increase in t causes an added influx of individuals who should have been "sex-ratio" but are now normal and they carry SR as well as sr genes. As time goes by the frequency of sr in "sex-ratio" individuals must go down so that the normals gain mainly Sr by this flow. Hence the rate of convergence α to unity decreases and the frequency p increases with increase in t.

4. Conclusions

In a randomly mating population of Drosophila of the normal and "sex-ratio" cytoplasmic types, it is shown that endogeneous oscillations in the fraction of normal animals may occur if the selective advantage of the abnormal cytoplasm decreases sharply as the fraction of females carrying it increases. A method is given for determining the stability or instability of any equilibria defined by equations (2.1), (2.2). The abnormals will be eliminated unless the product of the selective advantage of abnormals and the chance of passage of cytoplasmic particles is greater than unity.

When a gene is necessary for the continued multiplication of the cytoplasmic particles present in the abnormals, we have only studied in detail the case of constant selective pressure. Here the forces against the persistence of "sex-ratio" cytoplasm are again the possibility of no transmission of particles (measured by the probability t) but also now the possibility of no multiplication (measured by the fraction of sr alleles, s). The only possible force for the maintenance of "sex-ratio" cytoplasm is again its selective advantage. So we would expect that the gene-cytoplasm interaction would decrease the ultimate proportion of "sex-ratio" cytoplasm present and therefore increase the ultimate value of α the fraction of normal cytoplasm. This is in fact what happens. When the sr allele is initially absent we have seen above that S must be greater than $1/(1 - t)$ to prevent the extinction of the abnormal cytoplasm and that when this condition is met the fraction of abnormal cytoplasm has the limiting value

$$\frac{S(1 - t) - 1}{(1 - t)(S - 1)}. \tag{4.1}$$

When only sr is present, the abnormal cytoplasm is eliminated in one generation, whatever the values of S and t. When both sr and SR are present initially, there are several cases to consider. Firstly if $S > 2$, and $t < 1/2$, the limiting fraction β of abnormals is

$$\frac{S - 2}{S - 1}. \tag{4.2}$$

It is easily shown that the fraction (4.2) is less than the fraction (4.1) as would be predicted. Secondly if $S > 2$ and $t > 1/2$, the forces of selection and non-transmission are so strong that the presence of sr genes is not felt and the limiting value of β is zero if $S(1 - t) < 1$ and (4.1) when $S(1 - t) > 1$. When $S < 2$, any non-zero initial proportion of sr is sufficient to eliminate the abnormal cytoplasm, regardless of the value of t.

In the case when S is greater than 2 and t less than 1/2, the sr alleles tend to accumulate in the normal cytoplasm while, in the abnormal cytoplasm, the distribution of alleles will be nearer equality. In all other cases when the abnormal cytoplasm is not eliminated the whole population will ultimately contain no sr alleles; the forces have here acted so as to eliminate the sr allele and not the abnormal cytoplasm.

When the abnormal cytoplasm is eliminated, the ultimate gene-frequencies will depend on their initial levels and no formulae are available. The computations however give some idea of the movements to be expected.

Acknowledgments

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Summary

In several Drosophila species there are many more females than males. In some cases, this may be explained by assuming the existence of self-reproducing cytoplasmic particles, whose presence in the cytoplasm of a male is lethal. It appears that these particles are not always transmitted from mother to offspring and that their multiplication requires the presence of a gene. Furthermore females carrying these particles may enjoy a selective advantage. In the present paper, a mathematical study is made to determine the ultimate structure of such populations after many rounds of random mating. The main results are formulae for the proportions of individuals with normal and abnormal cytoplasms, and the frequency of the gene concerned, at equilibrium.

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NOTES AND COMMENTS

MUTANT GENES AND LINKAGE RELATIONSHIPS IN *DROSOPHILA PAULISTORUM* [1]

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Drosophila paulistorum, a member of the *willistoni* species group in the subgenus *Sophophora*, was described in 1943 by Dobzhansky and Pavan (Burla *et al.*, 1949). This species became particularly interesting since Dobzhansky and Spassky (1959) found that it is an aggregate of several subspecies or incipient species. This finding has raised numerous problems concerning the origin and the development of reproductive isolating mechanisms between incipient species. Genetic analysis of these isolating mechanisms cannot be carried out without having the chromosomes of at least some of the subspecies marked by at least one mutant marker gene each. It was for this purpose that we undertook to obtain and to locate in the chromosomes as many mutant genes as could be found in the species. Three subspecies were employed—the Centro-American, the Amazonian, and the Andean-South Brazilian. For information concerning the geographic distribution and the properties of these subspecies, see Dobzhansky and Spassky, 1959.

MATERIAL AND METHOD

Three strains have been employed to obtain mutants in the three subspecies of *D. paulistorum*. These strains were derived from wild populations collected, respectively, at Lancetilla, Honduras (the Centro-American subspecies), at Belem, Para, Brazil (the Amazonian subspecies), and at Angra dos Reis, state of Rio de Janeiro, Brazil (the Andean-South Brazilian subspecies).

The experiments were begun by exposing young males of a given strain to about 5,000 r-units of X-rays, crossing them to untreated females of the same strain, and examining the F_1 and F_2 generation progenies for mutants. It was found that with this high dose of the radiation a majority of the flies became sterile, and the mutants found in the progenies of the few fertile ones were too often associated with gross chromosomal aberrations, particularly with reciprocal translocations, which made the mutants useless for our purposes. The X-ray dosage was accordingly reduced to 3,000 r, and kept at that level in all subsequent experimental treatments. The treated males were outcrossed to untreated virgin females, using four pairs per culture. The F_1 flies were examined for autosomal and sex-linked dominant mutations. Small mass cultures of F_2 and F_3 generations were then made to find sex-linked and autosomal recessive mutants. Occasionally an F_4 progeny was also examined. Some apparently spontaneously arising mutants were also found during the experiments, but these were naturally few in number. All the experimental cultures were kept at room temperature.

SEX-LINKED MUTANTS

Centro-American group:

Texture (*Tx*)—in an X-rayed culture. Lethal in males, in heterozygous females producing a severe roughening of the eyes.

veinless (*vl*)—in an X-rayed culture. Recessive. All wing veins absent. Very poor viability in males.

yellow (*y*)—in an X-rayed culture. Although the wild type body color of the species is brownish-yellow, this mutant is easily distinguishable because of its very bright yellow body. Yellow males are sterile.

Amazonian group:

Notch (*N*)—in an X-rayed culture. Dominant, lethal in males. Wing veins thickened, producing small deltas at the margins. The notching is most pronounced at the ends of the third and fourth wing veins.

white (*w*)—spontaneous. Recessive. Eyes, testes and Malpighian tubules not pigmented. This is the best available sex-linked mutant in *D. paulistorum* since both males and females are fertile and viable.

Andean-South Brazilian group:

No sex-linked mutants were obtained either spontaneously or in X-rayed cultures despite repeated attempts to find them.

[1] This note is being published as background material to the article by Lee Ehrman, elsewhere in this issue.

[2] Permanent address: Faculdade Nacional de Filosofia, Universidade do Brazil, Rio de Janeiro. The work reported here was carried out during the tenure of a fellowship of the Rockefeller Foundation.

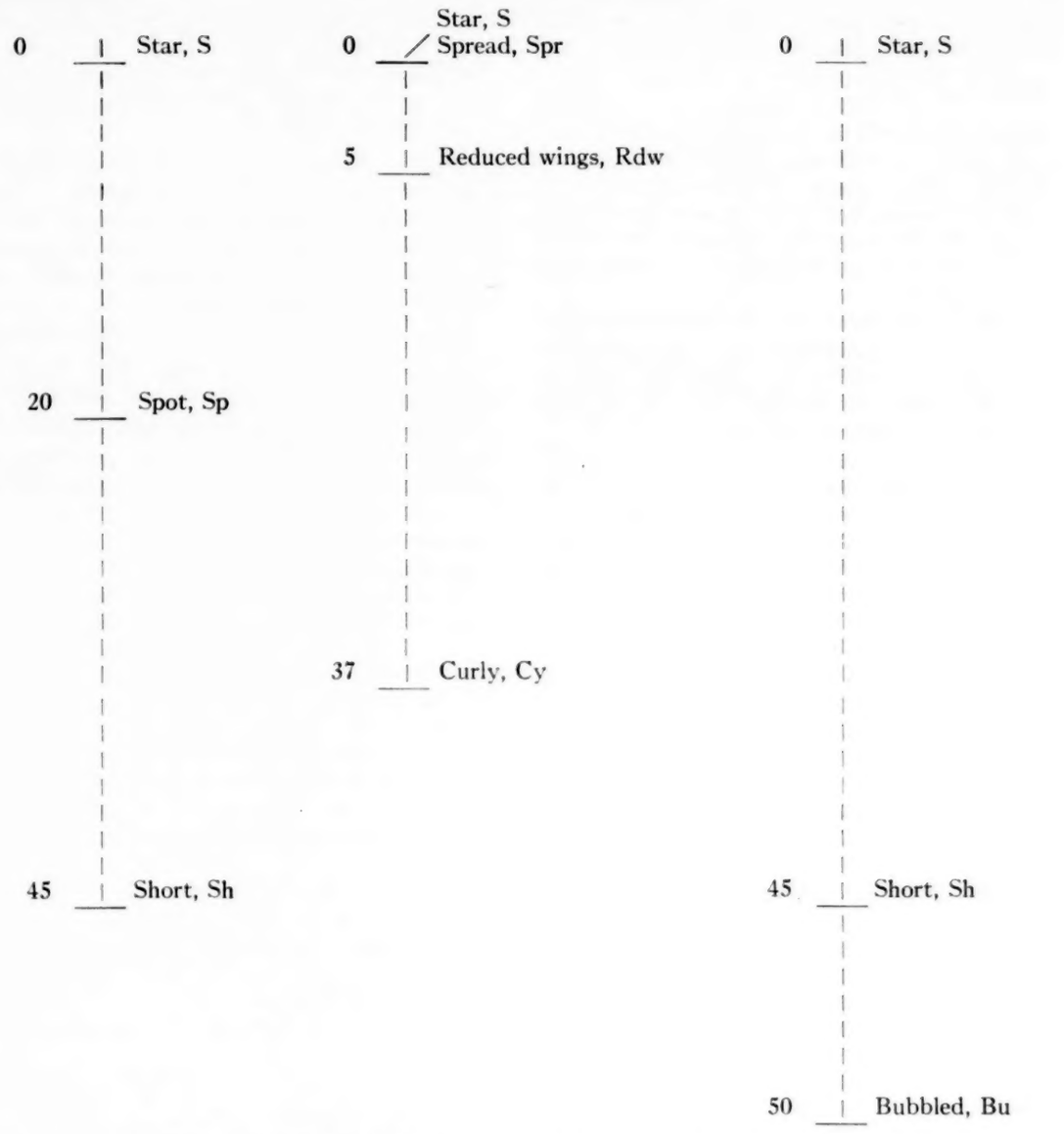

FIG. 1. Linkage maps of the second chromosomes of the Centro-American (left), the Amazonian (center), and the Andean-South Brazilian (right) groups of *Drosophila paulistorum*.

The authors must admit a striking lack of success with the X-chromosomes of the three subspecies; they proved unusually refractory to X-rays as a mutagen, although various dosages were tested. *Notch, yellow, veinless,* and *Texture* could not be judged "good" sex-linked mutants because of their effects on the males. Only the white-eyed mutation in the Amazonian group remains; it is an excellent analogue of *white* in *D. melanogaster.*

MUTANTS OF THE SECOND LINKAGE GROUP

Centro-American group:

Short (*Sh*)—in an X-rayed culture. Dominant, lethal when homozygous. The fifth vein does not reach the wing margin. Often, this vein extends no further than the posterior crossvein. (There is a probably non-homologous, but identically named mutant in *D. pseudoobscura.*)

Spot (*Sp*)—in an X-rayed culture. Dominant and quite constant in expression. Excellent viability in both sexes. This is a wholly new mutation, apparently without known parallels in other *Drosophila* species. The dorsal surface of the thorax is darkened and pinched laterally. This causes the wings to be tilted upward and easily broken. In some cultures *Spot* is found to be viable when homozygous.

Star (*S*)—in an X-rayed culture. Domi-

nant, lethal when homozygous. Eyes roughened because of irregularly arranged facets.

Amazonian group:

Curly (*Cy*)—in an X-rayed culture. Dominant, lethal when homozygous. Wings curved upward. The degree of curvature is very variable, especially when the stock is outcrossed to different wild strains. This mutation seems free of crossing-over suppression in this species.

Plum (*Pm*)—in an X-rayed culture. Dominant, lethal when homozygous. In young flies the eyes are mottled, but the color becomes uniformly dark as the flies age. *Plum* is a powerful crossing-over suppressor.

Reduced wings (*Rdw*)—in an X-rayed culture. Dominant, lethal when homozygous. The wings are about three-fourths of normal size, and are always held slightly spread outward from the body.

Spread (*Spr*)—in an X-rayed culture. Dominant, lethal when homozygous. Very poor viability because the extreme lateral spread of the wings causes the flies to become mired easily, especially in moist media. The fertility of the males is weak.

Star (*S*)—in an X-rayed culture. Dominant, lethal when homozygous. Homologue of *Star* in the Centro-American and the Andean-South Brazilian groups.

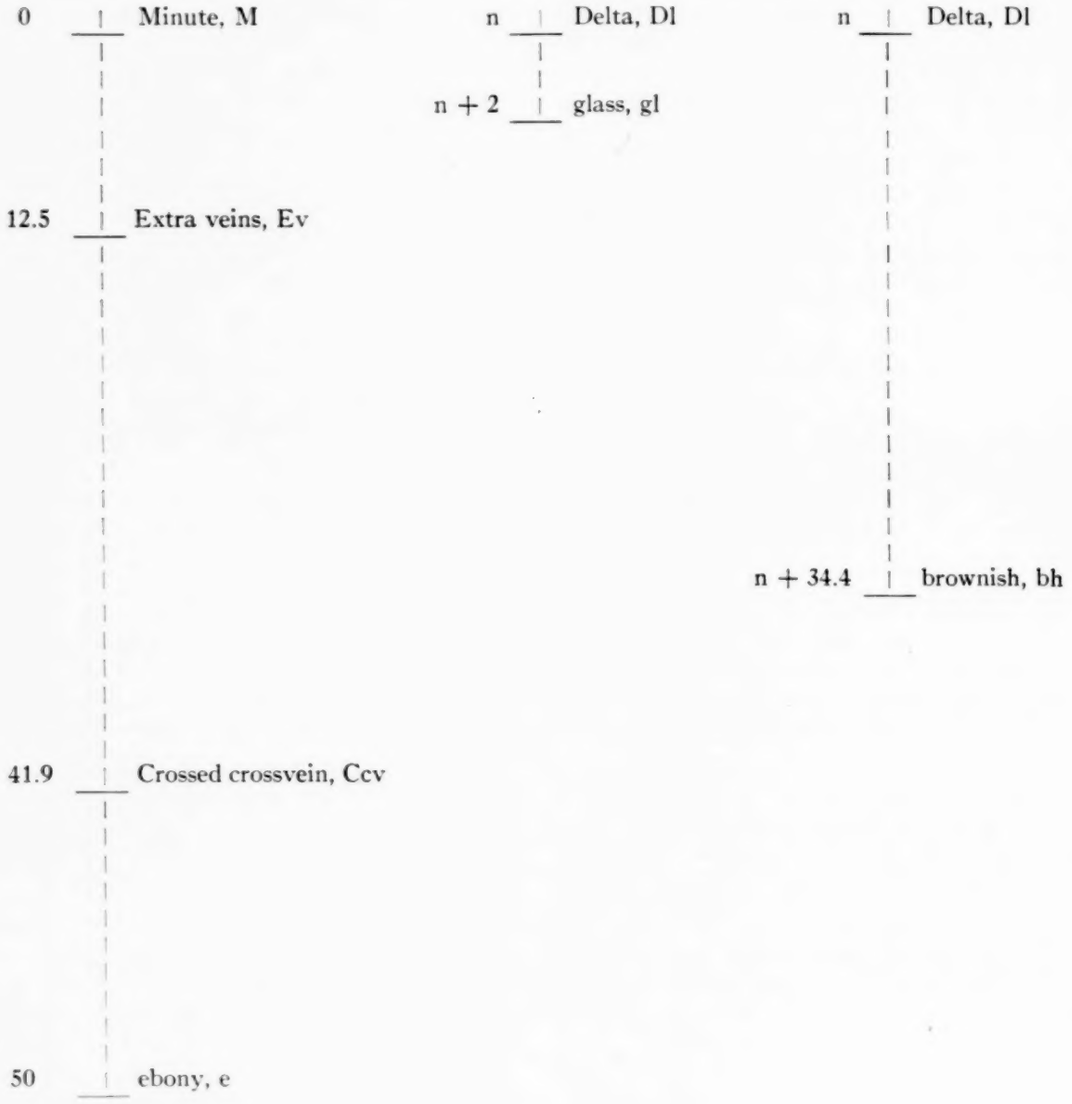

FIG. 2. Linkage maps of the third chromosomes of the Centro-American (left), the Amazonian (center), and the Andean-South Brazilian (right) groups of *Drosophila paulistorum*.

Andean-South Brazilian group:

Bubbled (*Bu*)—in an X-rayed culture. Dominant, lethal when homozygous. Wings severely blistered and inflated, veins thickened, and eyes roughened.

Short (*Sh*)—in an X-rayed culture. Dominant lethal when homozygous. Homologue of *Short* in the Centro-American group.

Star (*S*)—in an X-rayed culture. Dominant, lethal when homozygous. This is the only mutation which was induced in all three subspecies.

Mutants of the Third Linkage Group

Centro-American group:

ebony (*e*)—spontaneous. Recessive. Grayish-black body color, but not nearly as dark as *ebony* in *D. melanogaster*.

Extra veins (*Ev*)—in an X-rayed culture. Dominant, lethal when homozygous. Heterozygous flies have extra veins located between the longitudinal veins. Viability is poor, especially in males.

Minute (*M*)—spontaneous. Dominant, lethal when homozygous. Bristles reduced in size, especially the scutellar bristles. The developmental period is lengthened by about two days. Unlike many *Minutes*, the viability and fertility are good. This is fortunate since this is the only mutation affecting bristles acquired during this work.

Crossed crossvein (*Ccv*)—in an X-rayed culture. Dominant, lethal when homozygous. An extra section of wing vein is attached to the posterior crossvein so that it looks like a plus sign—"+." Eyes are slightly roughened. Very poor viability in both sexes.

Amazonian group:

Delta (*Dl*, Δ)—in an X-rayed culture. Dominant, lethal when homozygous. This mutation occurred frequently in X-rayed cultures of strains belonging to the Amazonian group. Wing veins thickened at the margins and crossveins. Eyes very rough.

glass (*gl*)—spontaneous. Recessive. Eyes reduced in size and very smooth. Eye color blotchy in both sexes, with patches of red and patches of creamy-white. Developmental period lengthened and viability reduced.

Andean-South Brazilian group:

brownish (*bh*)—spontaneous. Recessive. Unlike *brown* in *D. melanogaster* in that the eye color does not darken to garnet as the flies age, the testes are pigmented, and the linkage groups differ.

Delta (*Dl*, Δ)—in an X-rayed culture. Dominant, lethal when homozygous. This mutation occurred frequently in X-rayed cultures of strains belonging to the Andean-South Brazilian group as well as in strains of the Amazonian group. However, no *Delta* was ever observed in the Centro-American group.

orange (*or*)—spontaneous. Recessive. Somewhat lighter orange eyes in very young flies. By the time the flies are two days old the color is indistinguishable from the wild type of *D. paulistorum*. Thus, the value of this mutant as a marker is doubtful.

Discussion of the Linkage Maps

The chromosomal complement of *Drosophila paulistorum* consists of a pair of metacentric sex chromosomes (the X- and Y-chromosomes are indistinguishable at metaphase), a pair of metacentric second, and a pair of acrocentric third chromosomes. Microchromosomes are absent in this species. The three linkage groups found among the mutants therefore correspond to the three pairs of chromosomes.

The linkage maps of the second and third chromosomes in each of the three subspecies are presented in figures 1 and 2. Since the purpose of this work was to obtain genetic markers for the analysis of reproductive isolating mechanisms between the subspecies, mutations which impaired the fertility or viability of either sex were clearly not convenient for such experiments (see Ehrman, in this issue). With this object in mind, the authors did not calculate the map distance for a number of mutations which seriously impaired the capacities of the males which carried them. These stocks were eventually discarded. In the first linkage group, *Texture, veinless, yellow,* and *Notch* had to be eliminated, and, among the autosomal mutants, *Spread* (II) and *orange* (III) were, unfortunately, of no value.

The authors are aware that the chromosome maps presented here are, in no way, detailed enough to satisfy an interest in the chromosomes *per se*. This represents, nonetheless, the first attempt to bring this species under genetic control since it was described some sixteen years ago. Because *Drosophila paulistorum* is now known to represent, at our time level, a number of forms in a state of transition between race and full species, its response to a mutagenic agens was a necessary prerequisite for further genetic analysis.

Addendum

Since this article was submitted for publication, a new mutation arose spontaneously in the Belem, Para, Brazil stock (Amazonian subspecies). It was the *sex-linked* recessive, *vermilion*, and was subsequently found to be located 35 crossover units from *white* in the same subspecies. The new mutants are viable and fertile, and *"v"* seems to be an excellent

analogue of *vermilion* in *D. melanogaster*, (located 31.5 units from *white*).

Summary

The Centro-American, Amazonian, and Andean-South Brazilian subspecies of *Drosophila paulistorum* have three groups of linked genes corresponding to the haploid number of chromosomes in the species. With a relatively low (3,000 r-units) dosage of X-rays as the mutagen, chromosome maps could be drawn for each of the two autosomes. The scarcity of sex-linked marker genes precluded the possibility of the compilation of a map for the first linkage group.

Acknowledgments

The authors wish to acknowledge the use of the X-ray machine in the Carnegie Institute at Cold Spring Harbor, New York, and to thank Dr. Bruce Wallace for his assistance in treating the flies. Additional flies were irradiated by Mr. Monroe Strickberger and Dr. S. Hendler, Brooklyn. Advice about the compilation of the chromosome maps was received from Mr. Boris Spassky. Prof. Th. Dobzhansky kindly criticized the manuscript.

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EVOLUTIONARY FUNDAMENTALS OF PLANT BREEDING[1]

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Much of our knowledge of evolution originates from observations and investigation made by plant breeders working with cultivated plants, and it was no coincidence that Charles Darwin substantiated his theory of evolution by aid of such works. Plant breeding, which is among the oldest of botanical studies, has lately become somewhat overshadowed by the theoretical approaches it initiated, and at present many regard it more as a kind of applied cytogenetics than as an approach in its own right. But there is no doubt that plant breeding still is and always will be the most effective method of improving and increasing our food production, at the same time as it adds constantly to our knowledge of the processes of evolution.

Biosystematists and others working on the theoretical aspect of evolutionary processes tend to forget that recent contributions toward the understanding of these processes do not always originate from their own experiments, but are apt to result from studies involving the practical application of evolutionary principles to cultivated plants. Biosystematists, for instance, have not contributed much to the studies of mutation and selection in the past, although they realize that these processes are fundamental to evolution, whereas plant breeders are constantly adding to just this subject. Also, though utilization of many of the methods of plant breeders might greatly increase the output from some theoretical experiments on evolution, this is realized by rather few biosystematists with the deplorable result that the growth of their science is somewhat hampered by the lack of proper methods. It is likely that this is in part caused by the fact that very few biosystematists have ever had the opportunity to become acquainted with plant breeding and its methods.

Every year thousands of pages are printed about different phases of plant breeding in journals published in many languages. No single plant breeder can follow all this information, and it is still less likely that even a fraction of it will ever become directly available to the biosystematists. Therefore, reviews and manuals published within reasonable intervals are of immense value to those

[1] Kappert, H., and W. Rudorf (Editors, in cooperation with H. K. Hayes and A. Müntzing). 1958. Handbuch der Pflanzenzüchtung. Vol. 1. Grundlagen der Pflanzenzüchtung. 848 pp. Paul Parey, Berlin and Hamburg.

who must become acquainted with the recent results and new theories of this old and ever-growing approach.

A recent volume, the first in a series of six making up the second though in fact entirely new edition of the *Handbuch der Pflanzenzüchtung,* reviews the fundamentals of plant breeding in such a way as to make them easily available not only to the plant breeder but in an equally high degree to the evolutionist interested in experimenting on the basis of these fundamentals.

Though the papers by all fifteen contributors are of value for biosystematists and evolutionists alike, some are so to a greater degree than are others. Thus, the introductory chapter by Professor Kappert on natural variation will hardly be more than a short repetition for those who are familiar with Stebbins' (1950) classical book on *Variation and Evolution in Plants,* and some of the other topics are also necessarily repetitions as compared with easily available books. Still other chapters are, however, highly recommendable as reading for evolutionists and are likely to increase or widen their knowledge of methods available for application to their special problems.

It is difficult to say which chapter is most interesting, since all are well composed. A more concise and concentrated account of propagation methods than the one given here by Gustafsson and Nygren on only 32 pages, however, can hardly be made. The authors not only review very thoroughly the terminology and differentiation of amphimictic and apomictic phenomena, but also give elucidative information on pollination mechanisms, flower form variations, sexual dimorphism, self sterility factors, and even sex determination mechanisms, and also a thorough review of the embryological background of gametophyte and embryo development. All this would certainly fill more pages if other authors had written the account, but Gustafsson and Nygren are also able to add a concentrated review of their speciality, apomixis and vegetative reproduction, and still have five pages left for a discussion of the differences in frequency of polyploids and their causes. The present writer joins issue with them on this matter, since it was demonstrated in 1949 that there is no statistical background for the hypothesis by Gustafsson on the connection between life-form and polyploidy, but this only increases the usefulness of the chapter as stimulation for further discussion, and all opinions and results cannot be crammed into such a limited space.

Another chapter of great interest to evolutionists is that by Straub on chromosomes, chromosome sets, and polyploidy. He shows the connection between chromosomal variations and variations in genetical factors, and also indicates how changes in the chromosomes may affect the population structure and the essential mechanisms of isolation. Even Straub discusses polyploidy and its effects on the physiology of the plant, and shows its importance for survival and dispersal to new habitats.

Plasmatic inheritance has been studied most intensely by Michaelis, and he reviews its significance and the methods of study. It is evident that many dissimilar processes may be included under this collective term, but although much has already been done towards an understanding of these phenomena, the review indicates that this field of study still is wide open for inquisitive minds combining genetical and physiological approaches.

Population genetics is a subject that has contributed much to our present understanding of natural variation and selection, and its achievements are reviewed in a 49-page chapter by Brieger. Since this chapter demonstrates many of the methods and statistical approaches of plant breeders, it is likely to be very useful to those biosystematists who want to study the effects of mutation and selection on evolution, and also to those who want to learn about the effects of gene complexes affecting subspecific segregation. But although much seems to be known from this field already, there is apparently room for new studies, and even the theoretical and mathematical background for some of the discussions by Brieger may not always be as firmly established as it seems at first glance. Population genetics is a fascinating and evolutionarily important subject, the closer studies of which will greatly enhance the understanding of evolution at and below the species level. It is to be expected, for instance, that such studies on self pollinators may solve many of the riddles of classical taxonomy, although for these purposes new and more advanced statistical methods may well need to be invented.

The chapters on natural selection and selection breeding, by Roemer and Rudorf, cross breeding by Rudorf, specific and generic hybrids by Oehler, mutations and mutation breeding by Gustafsson and v. Wettstein, and polyploidy breeding by Müntzing, are all of immense interest to those studying evolution. These authors review in a very clear manner all the basic principles of selection and indicate how these can be used as a basis for much needed theoretical investigations on natural selection. Plant breeders know much about how artificial selection affects changes in a population, and they also have formulas to show how fast or slowly selection may change a certain

population in a certain direction. It is very likely the perusal of these chapters may prove an invaluable basis for those who want to study the action of natural selection on natural populations. These chapters plus the last one on biometrics are probably of greater interest to biosystematists than are all the others together, because so few experiments have as yet been made on natural selection in action under different environmental conditions.

All in all it appears clear that plant breeding can furnish biosystematists with methods and ideas of great value. It is through the reciprocal influence of the two related disciplines that they may be expected to evolve most effectively.

DARWIN, AND AFTER DARWIN [1]

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University of California, Berkeley

The Darwin-Wallace centennial in 1958 and the celebrations in 1959 commemorating the publication of the *Origin of Species* have been marked by numerous assemblies for the verbal presentation of reviews of the past century and have occasioned the production of thousands of pages of printed matter. The problem for the consumer is to distinguish the genuinely inspired offerings from those which are merely opportunistic. The rather diverse collection of six essays which Dr. P. R. Bell has edited [2] is somewhat uneven in quality but, in my opinion comes into the first category. The unevenness seems to bear some relation to the ages of the contributors, for the occasion seems to have demanded and obtained more from the younger men. At any rate, their chapters contain more which has not been said before.

The first essay is by the editor, Dr. Bell, and is based on Darwin's last book, *The Power of Movement in Plants.* If little attention is paid here to this chapter, it is not because of any lack of quality in the writing or in the extent of the literature coverage (189 titles are quoted) but because its main concern is with phototropism rather than with evolution.

In the second chapter, Dr. J. Challinor addresses himself to the bothersome inadequacy of the paleontological record at the time of the publication of the *Origin,* and then attempts to assay its present value. His conclusion is that, one hundred years later, "paleontology confirms the theory of evolution, but it does so, as it were, rather grudgingly. One might have hoped for something more thoroughly convincing." However, have not our requirements also changed during the century? Despite the continual increase in the numbers of fossils which have been described, have we not demanded that they be the historical record of greater *amounts* of evolution involving ever more subtle processes?

Challinor examines carefully the kinds of evolutionary evidence which may justifiably be sought in the paleontological record and then illustrates it with two kinds of survey amongst British fossil remains. First, using stratigraphy as the basis of arrangement, he examines the known contents of Cambrian and Cretaceous beds. Here the many groups, genera and species appear to be deposited with very little evidence of serial order. More promising for the documentation of at least micro-evolutionary continua is the second survey in which Challinor treats groups of fossils in biological order, endeavoring to discover evolutionary trends within them. Although he gives very few details, he is in the process of describing seemingly satisfactory series of this sort when the chapter suddenly comes to an end.

Dr. J. B. S. Haldane follows with a commentary on natural selection. As might be expected he leans less heavily upon the literature than do his colleagues. Despite this he contrives, in a single chapter, to survey Darwinism and Neo-Darwinism, as well as evolution by allopolyploidy, to provide a simple sketch of genetics and a mathematical treatment of phenotypic and genotypic selection, as well as to discuss evolutionary rates, the modification of instincts and the nature of genetic assimilation. Haldane's general evolutionary conclusions are those which are acceptable to a majority of contemporary evolutionists but it is particularly satisfying to have them supported by a summary of Kettlewell's excellent work on industrial melanism in British populations of *Biston betularia,* the Peppered Moth. In addition, Haldane has written what may be the wisest summing-up of the case for Darwinism: "The history of science makes it

[1] With apologies to George J. Romanes.

[2] Darwin's Biological Work; some aspects reconsidered. Edited by P. R. Bell, xiii + 343 pp., illustrated, $7.50. Cambridge University Press, London & New York. (37 shillings and sixpence in Great Britain.)

almost certain that facts will be discovered which will show that the theory of natural selection is not fully adequate to account for evolution. But the same history makes it extremely improbable that these facts will be in any way related to the criticisms at present made of it. The physics of Newton and Galileo have proved inadequate in several respects, and are being replaced by relativistic and quantum mechanics. These, however, are even further from the medieval physical theories than were the theories of Galileo and Newton."

The chapter by Dr. P. Marler on developments in the study of animal communication takes its theme from *The Expression of the Emotions in Man and Animals* and, in my opinion, is the most original and inspiring in the book. As Marler points out, Darwin's own work appears, for once, to have had very little effect upon his immediate successors and it is only relatively recently that the direct study of communications between animals in natural or semi-natural environments has been resumed on a significant scale. Thus, Marler, in addition to surveying the existing literature, is able to give us an extremely lucid account of his own studies of visual and auditory communication between animals, particularly birds. The selective values of different kinds of communication-signal are made abundantly clear; their origins may be an independent matter from their perfection through natural selection, but at all times they are directly related to community environment factors. This chapter is particularly well provided with clear plates and line drawings; elsewhere in the book the plates are not always successful in illustrating textual material.

Dr. H. L. K. Whitehouse provides the second botanical chapter, this time devoted to a subject which lies directly in the mainstream of evolutionary study. Under the heading "Cross and Self-Fertilization in Plants" he gives an excellent historical account of the study of heterosis and the outbreeding mechanisms by which it is continuously produced in many vascular plants. The nature and evolution of incompatibility systems receive special attention, particularly appropriately in view of Darwin's discovery of their existence in heteromorphic taxa and his pioneer survey of their occurrence in other groups.

A point which Whitehouse might have stressed more is that Darwin did not appreciate what is probably the greatest contribution of cross-fertilization to evolutionary success, the repeated production of new combinations of characters upon which natural selection can act, so that the chances of the ultimate production of more closely adapted types are greatly increased. For Darwin it was simply a matter of vigor and the maintenance of fertility.

The final chapter is an attempt by Dr. J. S. Wilkie to assess the originality of Darwin's evolutionary work (a favorite occupation of centennial celebrants), chiefly by considering it in relation to the contributions of Buffon and Lamarck. Wilkie recognizes the importance of Buffon's assiduous collection of data, particularly that concerned with the geographical distribution of animals, showing how this made possible the later evolutionary pronouncements by Lamarck. Nevertheless, Buffon was unable to formulate a *causal* theory of evolution satisfactory to himself and Wilkie considers that this kept him from firm adherence to the evolutionary idea in general.

It is in his interpretation of Lamarck's published views, however, that Wilkie delivers his strongest blow at traditional, text-book history. Lamarck's name is so consistently associated with the doctrine of the inheritance of acquired characters (published by him in 1801) that he is usually assumed to have looked upon it as the fundamental mechanism producing the progressive evolution which he discussed in his later works. Wilkie's analysis of the evidence, however, demonstrates convincingly that it was otherwise. For Lamarck the Deist, the grand march of evolution from simple to complex was the expression of a divinely inspired (but quite comprehensible) inherent tendency towards perfection (and greater resemblance to man). The production of "off-beat" types through the inheritance of acquired characters, including the products of use and disuse, served merely to complicate this picture. Darwin, incidentally, was aware of this duality in Lamarck's evolutionary concept, as he makes clear in the Historical Sketch which he added to the *Origin*.

Consequently, when the *Origin* was to be published, Darwin found himself in an intellectual environment where he was forced to concentrate first upon the presentation of a reasonable causal theory *before* setting out the general evidence for evolution. To be convincing to skeptical Victorian naturalists it was necessary, as Wilkie writes, to "exorcise the spectre of Deism" by stressing the lack of any empirically verifiable directional tendency in the process of natural selection. So it was, in the book, that the discussions of geological succession, geographical distribution and the mutual affinities of living beings, although they intruded early into Darwin's experience, were treated only in the concluding chapters.

In fact, Darwin himself could have been quoted in this context for, in the introduction to the *Origin*, he wrote "In considering the origin of species it is quite conceivable that a naturalist . . . might come to the conclusion that species had not been independently created, but had descended, like varieties, from other

species. Nevertheless, such a conclusion, even if well-founded, would be unsatisfactory, until it could be shown *how* the innumerable species inhabiting this world have been modified . . . ." The italics are mine.

It will be clear from this review that the several contributors to Dr. Bell's book have covered a great deal of ground in the 343 pages available to them. Perhaps they have covered too much. Nevertheless, the result is a volume which should be extremely useful as a source book to biologists who are not primarily evolutionists. For the specialist in evolutionary studies there may be only a minor amount of data which is not already available somewhere in the literature, but even the specialist must value the book for the extremely convenient manner in which the information is assembled and commented upon. For the same reason, the book should have a strong appeal to advanced biology students in the universities, and at least two of the chapters might be required reading for them. However, because it is a compilation of such value, it is a tragedy that the publishers have seen fit to issue it without an index.

ON THE POSSIBLY POLYPHYLETIC ORIGIN OF REPTILES

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F. R. Parrington (1958) has recently called attention to the striking similarity of the hyomandibular bone of crossopterygian fishes to the stapes of pelycosaurian reptiles. These homologous elements are basically alike in shape, size, position and attachments. Although advanced captorhinomorph cotylosaurs, such as *Captorhinus*, had a somewhat different kind of stapes, thinner and horizontal, the stapes of some of the more primitive captorhinomorphs apparently resembled that of the pelycosaurs (see figures of occipital regions of captorhinomorphs published by Watson, 1954). Partly on the basis of this resemblance of the stapes in these reptiles to the crossopterygian hyomandibular, and also on the basis of the quite different arrangement in labyrinthodont amphibians, where the stapes was relatively thin and was directed upwards towards the otic notch, Parrington has suggested that all of the reptiles were derived from a stock other than that of the labyrinthodont amphibians. This seems to him to be simpler than to believe that the distal end of the stapes migrated upwards towards the otic notch in the transition, crossopterygian fish to labyrinthodont amphibian, only to migrate downwards again in the transition, labyrinthodont to reptile. Another basis for Parrington's suggestion is his assumption that the place of the intertemporal bone of crossopterygian fishes was taken, in all early reptiles, by a lappet of the parietal bone.

Parrington's suggestion is refreshing. Quite possibly, the captorhinomorph cotylosaurs may have descended from other than a labyrinthodont stock. Such a descent, however, is not so easy to postulate for the diadectomorph cotylosaurs. For one thing, there are similarities between *Seymouria* and *Diadectes* that imply community of origin (Olson, 1947; Watson, 1954), and, whether *Seymouria* is regarded as an amphibian or as a reptile, it is certainly closely related to the labyrinthodont amphibians. Further, although the evidence is indirect, the stapedial apparatus in *Diadectes* must have been much like that in living reptiles (Watson, 1954, p. 391) and could not have been much different from that in the labyrinthodont amphibians. Even more significant, at least one diadectomorph reptile, the procolophonid *Nycteroleter* from the Russian Permian, had a basically labyrinthodont-like otic notch and occiput, and its relative, *Nyctiphruretus*, had a labyrinthodont-like occiput (Efremov, 1940). Watson (1951, p. 132) has already commented that it seems impossible that the occipital patterns seen in these procolophonids could have been secondarily derived from an occiput of the kind seen in captorhinomorph cotylosaurs. To this list of arguments may be added the possibility that an intertemporal bone did exist in *Diadectes*. Although Watson (1954) denies that there was such a bone in this genus, Olson has described an intertemporal bone in *Diadectes* (1947) and has discussed the variations of this bone in its outline and in its fusion with neighboring elements (1950); and G. E. Lewis and P. P. Vaughn are currently studying an immature diadectid, from the Cutler formation of Colorado, that shows evidence of the presence of an intertemporal bone.

Although they disagree on the relationships of the more advanced groups of reptiles, both Olson (1947) and Watson (1954) see basically different origins for the captorhinomorph cotylosaurs as opposed to the diadectomorphs. Is it possible that, even though the ancestry of

the captorhinomorph cotylosaurs may not have passed through a (typical) labyrinthodont amphibian stage, the ancestry of the diadectomorph cotylosaurs did? This suggestion involves a diphyletic origin of reptiles, and it presents several problems. The obvious problem is the lack of a record of suitable non-labyrinthodont amphibian ancestors for the captorhinomorphs. Parrington has constructed hypothetical "prototetrapod" and "protoamniote" ancestral stages for reptiles in general. Perhaps the ancestors of the captorhinomorphs were close relatives of the labyrinthodont amphibians in which the typical otic notch had not been developed; such an ancestry would account for the labyrinthodont-like occiput of the primitive captorhinomorph *Limnoscelis* (see Romer, 1946). Another problem is the seeming difficulty of polyphyletic attainment of the amniote stage. Perhaps this stage was reached earlier in tetrapod evolution than the attainment of the osteologically reptilian stage, but it may also be that the amnion was more easily duplicated than we have thought: Tavolga (1949) has described an amnion-like structure in the head region of an embryonic teleost fish. Also to be accounted for are the postcranial similarities of the diadectomorph and captorhinomorph cotylosaurs, but such similarities could be expected to have arisen during an early period of adaptation to a terrestrial environment by animals with a presumably roughly similar heritage of postcranial characters.

The suggestion of a diphyletic origin of reptiles may be compared with the currently widely accepted view of the polyphyletic origin of mammals from the therapsid reptiles (see Kermack & Mussett, 1958; Olson, 1959; Simpson, 1959). In recent years, our phylogenetic trees have been turned into bushes, and we see now that these bushes may have branched underground, with separate shoots breaking through the interface between structural grades. It is easy to accept the view of a polyphyletic origin of mammals, because there is a fairly good record of the transition from reptiles to mammals, but, where fossils are fewer, it is easier to construct simpler, monophyletic charts. What is needed is a much better record of the transition from amphibians to reptiles. Our record of Carboniferous reptiles is a scanty one, but we know that by late Pennsylvanian times there were already in existence: captorhinomorph cotylosaurs (Gregory, 1950; Peabody, 1959), a diadectomorph cotylosaur (Case, 1908), the three suborders of pelycosaurs (Peabody, 1957), and perhaps a diapsid reptile (Peabody, 1952). This diversity of known forms suggests an even greater diversity of Carboniferous reptiles yet to be discovered. Possibly the Carboniferous was to reptiles what the Mesozoic was to mammals: a time of polyphyletic origin and initial radiation, during which some of the finest achievements of the preceding structural grade had their heyday. Possibly, the origin was only diphyletic, resulting in the Parareptilia and Eureptilia of Olson (1947) or in the Sauropsida and Theropsida of Goodrich and Watson (see Watson, 1954; Vaughn, 1955), or there may have been more lines of origin.

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BOOKS RECEIVED

Notices will be given under this heading of books of evolutionary interest sent to the Editors. Appearance of a title in this section will not preclude a fuller review in a later issue of the journal.

Gale, Ernest F. 1959. Synthesis and Organization in the Bacterial Cell. vii + 110 pp. illus. $3.50. Wiley, New York.

Clausen, Robert T. 1959. *Sedum* of the Trans-Mexican Volcanic Belt: An Exposition of Taxonomic Methods. x + 380 pp. illus. $7.75. Comstock Publishing Associates, Ithaca, N. Y.

Penrose, L. S. 1959. Outline of Human Genetics. xii + 146 pp. illus. $2.50. Wiley, New York.